PRESENTED
BY
THE UNIVERSITY OF TORONTO
BY
Sir Henry Pellatt
Oct. 15th 1921

Digitized by the Internet Archive
in 2008 with funding from
Microsoft Corporation

http://www.archive.org/details/rothamstedmemoir01londuoft

Agric.

ROTHAMSTED MEMOIRS

ON

AGRICULTURAL CHEMISTRY

AND

PHYSIOLOGY.

(FROM THE 'PHILOSOPHICAL TRANSACTIONS.')

BY

SIR JOHN BENNET LAWES, BART., D.C.L., LL.D., F.R.S., F.C.S., &c.,

OF ROTHAMSTED, HERTS.,

AND

SIR JOSEPH HENRY GILBERT, M.A., PH.D., LL.D., F.R.S., V.P.C.S., &c.

VOLUME I.

CONTAINING REPORTS OF RESEARCHES

ON VEGETATION.

PUBLISHED 1861 AND 1889.

51063
7/9/01

LONDON:

PRINTED BY WILLIAM CLOWES AND SONS, LIMITED,

STAMFORD STREET AND CHARING CROSS.

1893.

ROTHAMSTED MEMOIRS.

CONTENTS OF VOL. I.

REPORTS OF RESEARCHES ON VEGETATION.

1. On the Sources of the Nitrogen of Vegetation, with special reference to the question whether Plants assimilate Free or Uncombined Nitrogen. (Philosophical Transactions, Part II., 1861, p. 431.*) 1861

2. On the Present Position of the Question of the Sources of the Nitrogen of Vegetation, with some new Results, and preliminary Notice of new Lines of Investigation. (Philosophical Transactions, Vol. 180, 1889, B., pp. 1-107) 1889

3.

* Reference to this paper will show, that the late Dr. Pugh, who took a prominent part in the investigation, was also a joint author.

ON

THE SOURCES OF

THE

NITROGEN OF VEGETATION;

WITH SPECIAL REFERENCE TO THE QUESTION WHETHER PLANTS ASSIMILATE FREE OR UNCOMBINED NITROGEN.

BY

JOHN BENNET LAWES, F.R.S., F.C.S.,

JOSEPH HENRY GILBERT, PH.D., F.R.S., F.C.S.,

AND

EVAN PUGH, PH.D., F.C.S.

From the PHILOSOPHICAL TRANSACTIONS.—PART II. 1861.

LONDON:

PRINTED BY TAYLOR AND FRANCIS, RED LION COURT, FLEET STREET.

1862.

REPRINTED BY SPOTTISWOODE & CO., NEW-STREET SQUARE.

1893.

XXIII. *On the Sources of the Nitrogen of Vegetation; with special reference to the Question whether Plants assimilate Free or uncombined Nitrogen.* *By* JOHN BENNET LAWES, *F.R.S., F.C.S.*, JOSEPH HENRY GILBERT, *Ph.D., F.R.S., F.C.S., and* EVAN PUGH, *Ph.D., F.C.S.*

Received June 21,—Read June 21, 1860.

CONTENTS.

PART FIRST.

GENERAL HISTORY, AND STATEMENT OF THE QUESTION.

	Page
SECTION I.—*Introduction, and early History*	433—435
SECTION II.—*Annual yield of Nitrogen per acre, in different Crops* :—	
A. Yield of Nitrogen per acre when the same crop is grown year after year, on the same land	435—438
B. Yield of Nitrogen per acre when Wheat is grown in alternation with Beans, or with Fallow	438—439
C. Yield of Nitrogen per acre when crops are grown in an actual course of Rotation	439—440
D. Relation of the increased yield of Nitrogen in the produce, to the amount supplied, when nitrogenous manures are employed	440—442
SECTION III.—*General view of the various actual or possible sources of the Nitrogen of our crops* :— Enumeration of, observations, &c.	442—447
SECTION IV.—*Review of the Researches of others, on the question of the assimilation of Free Nitrogen by Plants, and on some allied points* :—	
Introductory observations	447
A. The Experiments of M. BOUSSINGAULT	448—455
B. The Experiments of M. G. VILLE	455—463
Summary statement of the Results, and conclusions, of M. BOUSSINGAULT, and M. G. VILLE	463—464
C. The Experiments of M. MÈNE	464
D. The views of M. ROY	464—465
E. The Experiments of MM. CLOEZ and GRATIOLET	465
F. The Experiments of M. DE LUCA	465—466
G. The Experiments of M. HARTING	466
H. Experiments recorded by M. A. PETZHOLDT	467
Concluding observations	467—468

PART SECOND.

EXPERIMENTAL RESULTS OBTAINED AT ROTHAMSTED, DURING THE YEARS 1857, 1858, AND 1859.

Introductory observations	468—470
SECTION I.—*Conditions required, and Plan adopted, in Experiments on the Question of the assimilation of Free Nitrogen by Plants* :—	
A. Preparation of the soil, or Matrix, for the reception of the Plant, and of the nutriment to be supplied to it	470—472

Page

B. The Mineral Constituents added to the prepared soil 472
C. The Distilled Water 473
D. The Pots used to receive the Soil, Ash, Plant, &c. ..... 473—474
E. Final preparation of the Soil, Ash, and Pot, for the Plant 474
F. The Seeds taken for Experiment ..... 474—475
G. The Atmosphere supplied to the Plants ..... 475—476
H. The Apparatus used to enclose the Plants, and to supply them with Air, Water, Carbonic acid, &c. ..... 476—478
I. Use of the Apparatus ..... 478—480
J. The supply of Carbonic acid to the Plants ..... 480—481
K. Advantages of the Apparatus adopted ..... 481—483
L. Adaptation for healthy growth of the conditions of experiment adopted ..... 483—484

Section II.—*Other Conditions of Experiment requiring collateral Investigation* :—
Enumeration of 484
A. General considerations in regard to the possible influence of Ozone on the supply of combined Nitrogen to growing Plants ..... 484—486
B. Composition of the Gas in Plants ..... 486—495
C. Experiments on the action of Ozonized air on decomposing Organic Matter, and porous and alkaline substances ..... 495—497
D. Evolution of free Nitrogen in the decomposition of Nitrogenous organic matter ..... 497—508
E. Experiments on the action of the oxidizing and reducing forces, as manifested in the decomposition of Nitrogenous organic matter ..... 509—515
F. The mutual relations of Gaseous Nitrogen and the Nascent Hydrogen evolved during the decomposition of organic matter ..... 515—516

Summary Statement of the Results of the consideration of the conditions required, or involved, in Experiments on the question of the assimilation of free Nitrogen by Plants ..... 516—517

Section III.—*Conditions of growth under which assimilation of Free Nitrogen by Plants is most likely to take place. Direct experiments upon the Question under various circumstances of growth* :—
A. General consideration of conditions of growth ..... 517—520
B. Direct experiments on the question of the assimilation of free Nitrogen by plants 520—540
I. Experiments in which the plants had no other supply of combined Nitrogen than that contained in the seed sown ..... 521—529
II. Experiments in which the plants had a known supply of combined Nitrogen, beyond that contained in the original seed ..... 529—538
Relations of the plants grown with an extraneous supply of combined nitrogen to those grown without it ..... 538—539
Conclusions ..... 539—540

Summary of the Results of the whole Inquiry ..... 540—541

Appendix.

A. Preparation of solutions for manuring the plants, dates of application, and quantities applied ..... 542—543
B. Taking up the plants, preparation for analysis, methods of analysis, &c. ..... 543—547
C. Abstract of the Records of Growth of the Plants :—
I. Plants grown in 1857 ..... 548—558
II. Plants grown in 1858 ..... 558—577

Plates XII. XIII. XIV. and XV.

PART FIRST.

GENERAL HISTORY, AND STATEMENT OF THE QUESTION.

Section I.—INTRODUCTION, AND EARLY HISTORY.

The facts at the present time generally accepted regarding the ultimate composition, and the sources of the constituents, of plants, have, for the most part, received their preponderating weight of proof within the limits of the present century. But it is to the century preceding it that we must look for the establishment of much that was essential as the foundation of those advances which have since been made.

Whatever may be the value at present attached to the particular views of Hales regarding the composition and the sources of vegetable matter, we must accord to his labours, in the early part of the eighteenth century, the merit of having been guided by a proper spirit of experimental inquiry. Nor did he fail in applying to good account, and even in extending, the then existing knowledge of the material things around him which were apparently involved in the mysterious processes of vegetable growth.

With our present knowledge, however, of the general composition of plants, and of the sources of their constituents, it is easy to see how essential was a proper understanding of the chemistry of the air, and of water, to any true conceptions of the material changes involved in the vegetative process. It can, indeed, hardly excite surprise, that what may be called the germs of our present knowledge of the chemistry of plant-growth came forth almost simultaneously with the now adopted views of the composition of those universal, though not exclusive, media of vegetation—*air*, and *water*.

Accordingly, it is between the dates of 1770 and 1800 that we find Black, Scheele, Priestley, Lavoisier, Cavendish, and Watt establishing for us the facts that common air consists chiefly of nitrogen and oxygen, with a little carbonic acid, that carbonic acid itself is composed of carbon and oxygen ; and that water is composed of hydrogen and oxygen ; and it is within the same period that Priestley and Ingenhousz, Sennebier and Woodhouse, laboured to show the mutual relations of these bodies and vegetable growth.

But the observers last mentioned seem to have had more prominently in view the question of the influence of plants upon the media with which they were surrounded, than that of the influence of these media in contributing materially to the increased substance of plants themselves. Following closely on their footsteps, both in point of time and in general plan of research, came De Saussure. His labours were conducted towards the end of the last century and in the beginning of the present one ; and their results, and the arguments he founded upon them, published by him in 1804, may be said to have indicated, if not indeed established, many of the most important facts with which we are yet acquainted regarding the sources of the constituents stored up by the growing plant. To De Saussure we owe the experimental, and even quantitative, illustration of the fact, that plants in sunlight increase in their amounts of carbon, hydrogen, and oxygen, at the expense of carbonic acid and of water. It is remarkable, too, that, in the case of the main experiment he cites on the point, he, with his very imper-

fect methods, found the increase in carbon and in the elements of water to be almost identically in the proportion in which these are known to exist in the so-denominated carbo-hydrates. He further maintained the essentialness of the so-distinguished "mineral" constituents of plants; and he pointed out, in opposition to previous views, that they were derived from the soil, and were not the result of a creative power exercised by the living plant. He also called attention to the probability that the incombustible or mineral constituents derived by plants from the soil, were the source of those found in the animals which are fed upon them.

Besides carbon, hydrogen, oxygen, and the more peculiarly mineral constituents, plants had already been shown to contain *nitrogen*. Priestley and Ingenhousz thought they had observed that plants absorbed the free nitrogen of the confined atmospheres in which they were placed in their experiments. Sennebier and Woodhouse arrived at an opposite conclusion. De Saussure, again, did not find that plants took up appreciable quantities of the nitrogen supplied to them in the free and gaseous form. On the other hand, he thought that his experiments indicated rather an evolution of that element at the expense of the substance of the plant, than any assimilation of it from gaseous media. On this point he further concluded that the source of the nitrogen of plants was, more probably, the nitrogenous compounds in the soil, and the small amount of ammonia which he demonstrated to exist in the atmosphere.

From his results, as a whole, De Saussure concluded that air and water contributed a much larger proportion of the dry substance of plants, than did the soils in which they grew. In his view, the fertile soil was the one which yielded liberally to the plant nitrogenous compounds and the incombustible or mineral constituents; whilst he attributed to air and water, at least the main part of the carbon, hydrogen, and oxygen of which the greater portion of the dry substance of the plant was made up.

Up to the present time, carbonic acid and water are admitted to be the chief sources of the carbon, hydrogen, and oxygen which constitute the great proportion of vegetable produce. Nor is it questioned that ammonia, and especially ammonia provided within the soil, is at least an important source of the nitrogen of such produce. But the experiments of De Saussure—however sagacious his conclusions—were less satisfactory as to the source of the nitrogen, than as to that of the carbon, hydrogen, and oxygen, of vegetable matter.

It will not be supposed, from what has just been said, that there remain no questions of vast scientific as well as of practical interest, to be yet solved, regarding the conditions under which our different crops take up their carbon, hydrogen, and oxygen. At the same time, those who devote themselves to the subject of Agricultural Chemistry soon find that the explanation of the chemical phenomena of agricultural production awaits much more for a further elucidation of the sources, and of the modes of assimilation, of the *nitrogen* than of the other, so-called, organic elements of our crops—carbon, hydrogen, and oxygen.

In 1837, Boussingault took up the subject of the sources of the Nitrogen of plants, where De Saussure had left it more than thirty years before. To the investigations

and conclusions of BOUSSINGAULT, and others, in connexion with this question, from the date above mentioned up to the present time, we shall have to refer pretty fully further on. It may here be mentioned, however, that already at that early period BOUSSINGAULT had so far advanced in his inquiries into the chemical statistics of certain agricultural practices on the large scale, as to be apparently led by them to see the importance of investigating much more closely the sources of the Nitrogen periodically yielded by a given area of land, over and above that which was artificially supplied to it.

We fully admit the pertinence of the considerations, and the sagacity of the observations adduced on this head, more than twenty years ago, by BOUSSINGAULT. It will, nevertheless, be well to preface the discussion of our own experimental evidence regarding the sources of the nitrogen of plants, by the statement of a few prominent and striking facts, established by investigations conducted here, at Rothamsted, illustrative of the amounts of nitrogen yielded by different crops over a given area of land, and of the relation of these amounts to certain measured, or known, sources of it. Of these points, however, we profess to speak only in a very brief and summary manner on the present occasion. The discussion in detail, of the evidence relating to them, would indeed itself exhaust the limits of our Paper. Moreover, we have already treated of this subject in a separate form, elsewhere* ; and it is our intention to consider it much more fully at some future opportunity.

SECTION II.—ANNUAL YIELD OF NITROGEN PER ACRE, IN DIFFERENT CROPS.†

A.—*Yield of Nitrogen per acre when the same Crop is grown year after year on the same Land.*

The following Summary Table shows the average annual amounts of nitrogen yielded per acre, in the crops enumerated, when each was grown for a number of years consecutively on the same land, without manure.

TABLE I.

Description of Crop.	Dates of the Experiments.	Number of Years.	Average Annual yield of Nitrogen per acre, without Manure.
			lbs.
Wheat	1844—1859 inclusive	16	24·4
Barley	1852—1859 inclusive	8	24·7
Meadow Hay	1856—1859 inclusive	4	39·4
Beans	1847—1858 inclusive	12	47·8

There were obtained, then, in each of the Cereal crops (wheat and barley) about $24\frac{1}{2}$ lbs. of Nitrogen per acre, per annum, without manure. In the case of each of the crops the land was, in an agricultural sense, exhausted at the commencement of the

* British Association for the Advancement of Science, Leeds Meeting (1858), Section B.

† The results given in this Section have been revised, and in some cases the periods over which the estimates are taken extended, since the reading of the Paper.

experiment ; that is to say, it had been brought to such a condition by previous cropping, that, in the ordinary course of practice, it would be deemed necessary to supply manure to it before growing another corn-crop. It may be further remarked that in the case of the wheat there is as yet little, but in that of the barley more obvious indication of progressive decline in the annual yield.

The meadow-land yielded nearly 40 lbs. of Nitrogen per acre, per annum, or above one-half more than the exclusively Graminaceous crops, wheat and barley. The heterogeneous produce, meadow-hay, contained, however, a good deal of Trifolium, and other Leguminous plants, intermixed with the Grasses. To this fact is to be attributed, at least in great part, its comparatively high amount of Nitrogen. It should be observed, too, that the average is as yet taken over only four years.

The Leguminous crop (beans) has given, over a period of twelve years, an average of nearly 48 lbs. of Nitrogen per acre, per annum. The yield of Nitrogen in this *Leguminous* crop was, therefore, nearly twice as great as in the *Graminaceous* corn-crops. The bean and allied crops are, however, very subject to disease, especially when grown too frequently on the same land. It is, at least in part, owing to this circumstance, that the average annual yield over the twelve years was so much less than would be the yield of the crop when grown in suitable alternation with others in a season of average adaptation for its healthy development. In fact, so great was the deterioration in the character and amount of produce in the experiments in question, due to the continuous cropping, that whilst the average annual yield of Nitrogen over the first six of the twelve years was 70 lbs., that over the concluding six years was only 26 lbs. Nor did the addition of nitrogenous manure in the form of ammonia-salts, together with liberal mineral manuring, obviate this deterioration in any material degree more than did mineral manures alone.

In further illustration of the larger amount of Nitrogen obtained over a given area of land in Leguminous crops than in Graminaceous ones, some remarkable results with *clover* may be cited. Red clover was grown in three out of four consecutive years, the intermediate crop being wheat—all without manure. The following amounts of Nitrogen were obtained per acre :—

Table II.

Season.	Crop.	Nitrogen per acre.
		lbs.
1st Year, 1849	Clover.	206·8
(2nd Year, 1850 ...	Wheat.	45·2)
3rd Year, 1851	Clover.	29·3
4th Year, 1852	Clover.	111·9
Average of the three years Clover		116·0

All further attempts to grow clover year after year, on this land, have, however, failed. Neither ammonia-salts, nor organic matter rich in carbon as well as other constituents, nor mineral manures nor a mixture of all has availed to restore the

clover-yielding capabilities of the land. On the other hand, it should be particularly observed that, after taking 206·8 lbs. of Nitrogen from an acre of land in the clover-crop of the first year, the wheat-crop of the second or succeeding year, compared with that of the same season in the adjoining experimental wheat-field where the crop is grown year after year on the same land, was about double that obtained from the plot which had there been unmanured for a series of consecutive years, and fully equal to that from a plot which had for the same period received annually a dressing of farm-yard manure. It should be added that, after failing to get any crop of clover at all in 1853 and in 1854, and getting a very poor one in 1855, the land was allowed to lie fallow for two years; that after this, in 1858, there was obtained an over-luxuriant and laid crop of barley, more than twice as great as the average annual produce of eight years of the successive growth of the crop without manure in the same field; yet, after resowing with clover in the spring of 1859, and getting a small cutting in the autumn of the same year, the plant has again died off during the winter of 1859–60. This was the case notwithstanding that it was a perennial variety that was last sown.

Again, eight consecutive crops of *turnips* (four "White Globe" and four "Swedish") gave an average annual yield, per acre, of about 40 lbs. of Nitrogen, without the supply of any in the manure. In the case of these turnips, however, the land received annually certain "mineral" manures. In fact, turnips grown year after year without manure of any kind, yielded, after a few years, only a few hundred-weights of produce per acre; but the *percentage* of Nitrogen in these diminutive unmanured turnips was very unusually high. It will be observed that the average annual yield of Nitrogen per acre, in the turnips grown by mineral manures (containing no Nitrogen), was considerably more than that in the unmanured Cereal grain crops. And, in connexion with this point, it is worthy of remark, that, on barley, without manure, succeeding on the land from which these eight mineral-manured turnip-crops had been taken, the produce was only about three-fourths as much as that obtained, in the same season, where barley was grown for the second year in succession without manure, in another field; and it was only about three-fifths as much as that obtained, also in the same season, where barley was grown as the second crop of the second course, in a series of entirely unmanured four-course Rotation-crops.

It may be mentioned that, in the case of the purely Graminaceous crops, there has been but very little gain in the annual yield of Nitrogen per acre by the use of mineral or non-nitrogenous manures. But in the case of the Leguminous crops, as in that of the root-crops just referred to, there has been much more Nitrogen harvested over a given area, within a given time, when mineral manures were employed, than when no manure at all was used.

It has thus far been seen, then, that the Leguminous crops yield much more Nitrogen over a given area than the Graminaceous ones, and, further, that the amount of Nitrogen harvested in the former is increased considerably by the use of "mineral" manures, whilst that in the latter is so in a very limited degree. It is, nevertheless, a well-known agricultural fact, that the growth of the *Leguminous* crops, which *carry off* such a com-

paratively large amount of Nitrogen, is one of the best preparations for the after-growth of wheat. On the other hand, it is equally true that *fallow*—one important effect of which is to *accumulate* within the soil the available Nitrogen of two or more years for the growth of one—and *adding nitrogenous manures*, have each much the same effect in increasing the produce of the Cereal crops.

B.—*Yield of Nitrogen per acre when Wheat is grown in alternation with Beans, or with Fallow.*

The striking and interesting fact, that the growth (and removal from the land) of a highly nitrogenised Leguminous crop, and fallow, have each the effect of increasing the amount of produce, and with it the yield of Nitrogen per acre, of a succeeding Cereal crop, is briefly illustrated by the summary of direct experimental results given in the following Table :—

Table III.

Showing the Amount of Nitrogen obtained per acre, in Wheat grown consecutively, in Wheat alternated with Beans, and in Wheat alternated with Fallow.

Period of Experiment ten years, 1850—1859 inclusive.

		Nitrogen per acre, lbs.	
		Total.	Average annual.
Beans—10 crops consecutively	without Manure	346·9	34·7
	with Mineral Manure	510·6	51·1
Wheat—without Manure	10 Crops consecutively	234·0	23·4
	5 Crops alternated with Fallow	219·3	43·9 or 21·9
Wheat / Beans — without Manure	5 Crops alternated with Beans	225·8	45·2 or 22·6
	5 Crops alternated with Wheat	244·5	48·9 or 24·5
Wheat / Beans — with Mineral Manure	5 Crops alternated with Beans	207·0	41·4 or 20·7
	5 Crops alternated with Wheat	227·2	45·4 or 22·7

It is seen, then, that ten consecutive crops of beans, without manure of any kind, gave an average annual yield of Nitrogen, per acre, of 34·7 lbs.; and ten consecutive crops with "mineral" but without nitrogenous manure gave an average annual yield, per acre, of 51·1 lbs.

During the same period, ten consecutive crops of wheat without manure of any kind gave annually 23·4 lbs. of Nitrogen, or less than half as much as the beans with mineral but without nitrogenous manure. Again, extending over the same series of years, five crops of wheat *alternated with fallow* gave, taking the average of the five years under crop, 43·9 lbs., and on the average of the ten years, 21·9 lbs. per acre, per annum, of Nitrogen. That is to say, the wheat alternated with fallow gave, taking the average of the five years of its growth, nearly twice as much Nitrogen annually as the wheat grown after wheat in the same seasons. The total Nitrogen obtained, per acre, over the ten years, was, however, pretty much the same in the two

cases,—namely, 234 lbs. in the ten crops of wheat grown consecutively, and 219·3 lbs. in the five crops of wheat alternated with fallow.

Again, five crops of wheat *alternated with beans* gave 45·2 lbs. of Nitrogen per acre, per annum, over the five years—equal half that amount, or 22·6 lbs., averaged over the ten years. The total amount of Nitrogen obtained during the ten years was, in the ten crops of wheat grown consecutively, 234 lbs., in the five crops of wheat alternated with fallow, 219·3 lbs., and in the five crops of wheat alternated with beans, 225·8 lbs. —or not very materially different in the three cases. But, notwithstanding that the land has thus yielded in wheat, over ten years, almost as much total Nitrogen in five crops alternated with beans, as in ten crops grown consecutively, and rather more than in five crops alternated with fallow, the five intermediate crops of beans have, in addition to this, themselves carried off more than the same amount of Nitrogen as the wheat—namely, 244·5 lbs.

The general result is, then, that pretty nearly the same amount of Nitrogen was taken from a given area of land in wheat, in ten years, whether ten crops were grown consecutively, five crops in alternation with fallow, or five crops in alternation with beans. In fact, the crop of wheat was increased fully as much when it succeeded *beans*, which carried off a large amount of Nitrogen, and of mineral matters also, as when it succeeded *fallow*, which conserved the stores both of Nitrogen and of mineral matter.

It will be seen, by the illustrations given in the next sub-section (C), that the experimental results thus far adduced are perfectly consistent in character with those obtained under circumstances more nearly allied to those of ordinary farm practice.

C.—*Yield of Nitrogen per acre when crops are grown in an actual course of rotation.*

In Boussingault's experiments, he obtained, taking the results of six separate courses of rotation, an average of between one-third and one-half more Nitrogen in the produce than had been supplied in the manure. He found, moreover, that the largest yields of Nitrogen were in the Leguminous crops, and, further, that the Cereal crops were the larger when they next succeeded upon the removal of the highly nitrogenized Leguminous crops.

For our own experiments at Rothamsted upon an actual course of rotation, a piece of land was selected which was, in an agricultural sense, exhausted; that is to say, it had grown a course of crops since the application of manure, and would, under ordinary practice, have received a new supply before growing another crop. On this land the four-course rotation of Turnips, Barley, Leguminous crop (or Fallow), and Wheat, in the order of succession here enumerated, and *without manure*, has now been followed for twelve years—that is, through three separate courses. The yield of Nitrogen during these twelve years, or three courses, has been determined; and the result shows an average annual amount, per acre, of 42·6 lbs.* This, it will be remembered, is nearly

* The second and third courses gave, however, much less than the first, and hence a less average per annum than that stated for the twelve years.

twice as much as was obtained in either wheat or barley when these crops were, respectively, grown year after year on the same land. The greatest yield of Nitrogen obtained in the Rotation experiment was in the case of a clover-crop, grown once during the twelve years, and which constituted the Leguminous crop of the first course. After both this clover-crop (in which was removed such a large amount of Nitrogen) and beans which replaced it in the second and third courses (but which gave a very small yield of Nitrogen), the wheat-crop was about double as much as the average where wheat has been grown succeeding wheat, and it was about equal to the average per crop when wheat was grown after fallow, or after beans, in the experiments already referred to.

It has been seen, then :—that even Cereal crops grown, year after year, on the same land, gave an average of about 24½ lbs. of Nitrogen per acre, per annum ; that, under similar circumstances, Leguminous crops gave much more ; that, nevertheless, the produce of the Cereal crop was nearly doubled when it was preceded by the more highly nitrogenized Leguminous crop ; that the produce of the Cereal crop was also nearly doubled when it was preceded by fallow ; and lastly, that in an actual rotation of crops, though entirely without manure, there was also an average annual yield of Nitrogen nearly twice as great as that obtained in the continuously grown Cereal.

It has been incidentally mentioned, too, that the highly nitrogenous Leguminous crops are comparatively little benefited by the direct application of nitrogenous manures (ammonia-salts). It has also further been stated, on the other hand, that, notwithstanding the comparatively small amount of Nitrogen harvested in a Cereal crop, and that both the crop and its Nitrogen are very much increased when succeeding upon the growth and removal of a highly nitrogenous Leguminous crop, yet the application of nitrogenous manures is also one of the surest means of increasing the produce, and the yield of Nitrogen, of a Cereal crop.

D.—*Relation of the increased yield of Nitrogen in the produce, to the amount supplied, when nitrogenous manures are employed.*

Not only do we harvest in our crops (particularly the Leguminous ones) a large amount of Nitrogen, the source of which, it will afterwards be seen, is by no means fully explained, but, when we increase their growth (particularly that of the Cereals) by the direct application of nitrogenous manures, it is found that, over a series of years, a considerable proportion of the so-supplied Nitrogen is not recovered in the increase of crop.

Thus, when a certain amount of ammonia-salts (in addition to a complex mineral manure) was applied, year after year, for the growth of wheat, the result, taken over a period of six years, was, that the increased yield of Nitrogen in the crop was only equal to about 43 per cent. of the Nitrogen which had been supplied in the manure. When double the amount of ammonia-salts was employed, by which the crop was still further increased, the proportion of the supplied Nitrogen which was recovered as increase was almost identically the same ; but with more still, the proportion was less.

Again, when the smaller amount of ammonia-salts was applied annually, for six years,

to barley, the increased yield of Nitrogen corresponded to only about 42 per cent. of the Nitrogen supplied in the manure; and when the double amount of the manure was employed for barley, over the same series of years, only about 43 per cent. of the supplied Nitrogen were recovered as increased yield.

To the statement of these facts it should be added that the Nitrogen (equal in amount to, say 60 per cent. of that supplied in the manure) which is not obtained as increased yield in the immediate crop does not appear to exist in the soil availably for an immediately succeeding crop. Thus, when by the use of nitrogenous manures an increased yield of Nitrogen has been obtained in the first succeeding wheat-crop, equal in amount to about 40 per cent. of the Nitrogen supplied in the manure, the increased yield obtained in the second crop, without any further supply, is equal to little more than one-tenth of the remainder.

In connexion with this subject it may be mentioned that, so far as our experiments with *meadow-grasses* at present show, it does not appear that the increased yield of Nitrogen in the crop on the use of nitrogenous manures bears a much higher proportion to the amount supplied in their case than in that of either wheat or barley. In the case of the Leguminous corn-crops, the proportion of the increased yield to the amount supplied appears to be even less than in that of the Cereal grains. Root-crops, on the other hand, would seem to gather up an increase of Nitrogen bearing a larger proportion to the quantity directly supplied in the manure.

On the assumption that the relation of the immediately increased yield of Nitrogen to the amount supplied in manure represents really or approximately the proportion of the directly supplied Nitrogen which is actually recovered in the immediate crop, the following questions seem to suggest themselves:—

Is the unrecovered amount of supplied Nitrogen, or at any rate a considerable proportion of it, drained away and lost?

Are the nitrogenous compounds transformed within the soil, and their Nitrogen, in some form, evaporated?

Does the missing amount for the most part remain in some fixed combination in the soil, only to be yielded up, if ever, in the course of a long series of years?

Is ammonia itself, or Nitrogen in the free state, or in some other form of combination than ammonia, given off from the surface of the growing plant? Or, lastly,

When Nitrogen is supplied within the soil for the increased growth of the Graminaceous crop, is there simply an unfavourable distribution of it, considered in relation to the distribution of the underground feeders of the crop?—the Leguminous crop, which alternates with it, gathering from a more extended range of soil, and leaving a residue of assimilable Nitrogen within the range of collection of a next succeeding Cereal one?

But other and wider questions than those just enumerated present themselves on a careful review, as a whole, of the Nitrogen-statistics of field-produce to which attention

has briefly been directed. For the moment, all may be asked in one—namely, What are the sources of all the Nitrogen of our crops beyond that which is directly supplied to the soil by artificial means? This brings us to a consideration of the next Section of our subject.

Section III.—General view of the various actual or possible sources of the Nitrogen of our crops.

The following actual or possible sources of the Nitrogen obtained in our crops, beyond that supplied in manure, may be enumerated :—

1. The Nitrogen in certain constituent minerals of the soil, especially the ferruginous and aluminous ; and certain nitrides.

2. The combined Nitrogen annually coming down in the aqueous depositions from the atmosphere :—

   (*a*) As ammonia.
   (*b*) As nitric acid.
   (*c*) As organic corpuscles, &c.

3. The accumulation by the soil of combined Nitrogen from the atmosphere :—

   (*a*) By surface absorption aided by moisture.
   (*b*) By the chemical action of certain mineral constituents of the soil.
   (*c*) By the chemical action of certain organic compounds in the soil.

4. The formation of ammonia in the soil, from free Nitrogen, and nascent Hydrogen (the so-formed ammonia either remaining as such, or being oxidated into nitric acid).

5. The formation of nitric acid from free Nitrogen :—

   (*a*) By electric action.
   (*b*) With common Oxygen, in contact with porous and alkaline substances.
   (*c*) Under the influence of Ozone, or nascent Oxygen.

6. The direct absorption of combined Nitrogen from the atmosphere, by plants themselves.

7. The assimilation of free Nitrogen by plants.

A careful consideration of the above actual or possible sources of the Nitrogen of the vegetation which covers the earth's surface will show, in regard to some of them, that they at least are quantitatively inadequate to supply the amounts of Nitrogen which direct experiment has shown to be removable in various crops from a given area of land.

(1) The combined Nitrogen that may be due to certain of the constituent minerals of the débris of which our soils are made up cannot be supposed to be an adequate source of the nitrogen annually carried off in the vegetable produce of the land.

(2) The combined Nitrogen which comes down from the atmosphere in the various aqueous deposits of rain, hail, snow, mists, fog, and dew—whether it be merely the return from previously existing generations of plants or animals elsewhere, or whether in part the product of a new formation—undoubtedly does contribute materially to the

annual yield of Nitrogen in our crops. The amount of Nitrogen derivable from these sources is, moreover, perhaps more readily quantitatively estimated than that from any of the other sources enumerated. Accordingly, much labour has, of late years, been bestowed in determining the amounts of ammonia and nitric acid in these several aqueous deposits. Extensive series of observations have been made on these points by Boussingault, Barral, Way, and two of ourselves: and others have experimented on a more limited scale. It may be stated, generally, that the rain of the open country has indicated an average of very nearly the same amounts of ammonia in the hands of Boussingault in Alsace, and of Way and ourselves in England. The most numerous and reliable determinations of the amount of nitric acid in rain-water are probably those of Mr. Way.

By the aid of numerous determinations of the ammonia by ourselves, and of both the ammonia and nitric acid by Mr. Way, we are enabled to form an estimate of the total amount of Nitrogen coming down as ammonia and nitric acid in the total rain, hail, and snow, and in some of the minor aqueous deposits, during the years 1853, 1855, and 1856, here at Rothamsted, where the experiments relating to the acreage yield of Nitrogen in the different crops were made. The result was, that in neither of the three years did the Nitrogen so coming down as ammonia and nitric acid amount to 10 lbs. per acre.

Supposing the combined Nitrogen coming down in the direct aqueous deposits were to be estimated, in round numbers, at 10 lbs. per acre, per annum, this amount would supply less than half as much Nitrogen as was annually removed in the continuously grown wheat and barley crops. It would amount to only about one-fourth of that which was obtained in the hay, and in the turnips; to a less proportion of that obtained in beans; and to a still less proportion of that obtained in the clover. Lastly, it would amount to only about one-fourth as much as was obtained per annum, over twelve years of ordinary Rotation, but without manure of any kind either during that period or for some years previously.

We are driven, then, to seek for other sources of the Nitrogen of our crops, than that which comes down as ammonia and nitric acid in the more direct and more easily measurable aqueous deposits from the atmosphere. Nor does it appear, so far as can be judged from the results of Boussingault on this point, that the amounts of combined Nitrogen deposited by dew are such as to lead to the supposition that our approximate estimate would require any material modification, were as large a proportion of dew included in our collected and analysed aqueous deposits as is probably received by the soil itself or the vegetation which may cover it.

(3) With regard to the amounts of combined Nitrogen accumulated by the soil from the atmosphere by virtue of surface absorption, or chemical action, it is probable that they constitute no inconsiderable proportion of that which is annually available for vegetation over a given area of land. Numerous investigations have indeed been undertaken during the last few years, both by ourselves and others, to determine the actual or relative capacities for absorption of different soils, or constituents of soils. Unfortu-

nately, however, even quantitative results established by laboratory methods do not admit of very direct and certain application in accounting, quantitatively, for the amount of combined nitrogen that may be so fixed, to a given depth, over a given area of land, within a given time. We hope, however, to treat of this subject in some detail on some future occasion.

(4 & 5) The circumstances of the formation of ammonia, or nitric acid, from gaseous, dissolved, or nascent Nitrogen, are at present involved in too much obscurity, and are the subject of too much conflicting statement for their consideration to serve us much in our present inquiry. The various assumed actions are, as yet, by no means all clearly established in a merely qualitative way; and still less, quantitatively. Moreover, as in the case of absorption, so in that of the formation of ammonia, or of nitric acid, there would be considerable difficulty and uncertainty in applying the results of laboratory experiments to the estimation of the probable amount of the Nitrogen of vegetation due to such sources. To some of the questions involved, we shall, however, have to refer more or less in detail in discussing the conditions of the experiments which will form the subject of the second part of the present Paper.

(6) With regard to the direct absorption of ammonia or nitric acid from the air by plants themselves, we have little of either qualitative or quantitative evidence of any kind to guide us. Still, a few observations may be usefully hazarded, in passing, which may bear more or less directly upon the point.

In our ripened Cereal crops, we find 1 part of Nitrogen to somewhere about 30 parts of carbon; and in our Leguminous crops, 1 part of Nitrogen to about 15 or fewer parts of carbon. It is supposed that the atmosphere, on the average, contains 1 part (or rather more) of carbon in the form of carbonic acid to 10,000 parts of air. We may perhaps assume, as an extreme amount, that the atmosphere contains only 1 part of Nitrogen in the form of ammonia to about 12,000,000 parts of air. Adopting these assumptions, there would obviously be, instead of only 30 or 15 times less Nitrogen than carbon (as in the respective crops), 1200 times less Nitrogen in the ambient air in the form of ammonia, than of carbon in the form of carbonic acid in the same medium.

If, however, we were to adopt as more nearly the amount of ammonia in the air that found by M. G. Ville (namely, only about one-fifth as much as we have assumed above), it would then appear that there were 6000 times less of Nitrogen in the air in the form of ammonia, than of carbon in that of carbonic acid.

Taking the former or more favourable assumption of the two, the result would be that the ambient atmosphere contained Nitrogen as ammonia, to carbon as carbonic acid, in a proportion 40 times less than that of Nitrogen to carbon in the Cereal produce, and 80 times (or more) less than that of Nitrogen to carbon in the Leguminous produce. Adopting M. G. Ville's estimates, on the other hand, the proportion of the so-combined Nitrogen to the so-combined carbon, in the air, would be 200 times less than that of the Nitrogen to the carbon in the Cereal crops, and about 400 times less than that of the Nitrogen to the carbon in the Leguminous crops.

Looking, therefore, at the subject from the point of view of *actual quantity merely*, the ammonia in the atmosphere would appear very inadequate to yield Nitrogen in a degree at all corresponding to the yield of carbon by carbonic acid. It would appear too, from the observations hitherto recorded bearing upon the point, that the amount of Nitrogen existing in the atmosphere as nitric acid is very much less than that existing as ammonia. Hence, the inclusion, in the estimate of the combined Nitrogen in the atmosphere, of the amount existing as nitric acid would, in point of quantity, by no means materially affect the question.

But it is worthy of remark, in reference to the question of the proportion of Nitrogen as ammonia to carbon as carbonic acid, that may be available to vegetation from atmospheric sources, that, although the actual amount of Nitrogen as ammonia in the atmosphere is very small compared with that of the carbon as carbonic acid, yet, a given amount of water would absorb very much more Nitrogen as ammonia, or dissolve very much more Nitrogen as carbonate of ammonia, than it would absorb of carbon in the form of carbonic acid under equal circumstances. In illustration, it may be mentioned that water at 60° F. (about 15°·5 C.) would at the normal pressure absorb about 850 times as much Nitrogen in the form of ammonia as it would of carbon in the form of carbonic acid; and, under equal circumstances, very many times more Nitrogen as carbonate or even as bicarbonate of ammonia would be dissolved, than there would be of carbon as carbonic acid absorbed. There would appear to be, then, a compensating quality for the small actual amount of Nitrogen as ammonia in proportion to carbon as carbonic acid in the atmosphere, in the greater absorbability or solubility of the compounds in which Nitrogen exists than of the carbonic acid in which the carbon is presented. How far, however, the compensating quality here suggested may really influence the proportion of the Nitrogen to the carbon available from the atmosphere, in the combined form, under the actual conditions involved in vegetation, is a question the numerous and intricate bearings of which we do not profess here to enter upon.

Before passing from this question of the direct absorption of Nitrogen in the combined form from the atmosphere by plants themselves, one or two further observations may yet be made which are suggested by the actual facts of agricultural production. It is undoubtedly the case that the Graminaceous crops depend very materially upon combined Nitrogen *within the soil*, to determine the amount of their produce. They seem, however, to be comparatively independent of carbonic acid yielded by manure within the soil. The Leguminous crops, on the other hand, appear to be much less benefited by direct supplies of characteristically nitrogenous manures. It would hence seem that they are more able to avail themselves of Nitrogen supplied in some way by the atmosphere, possibly by the aid of their green parts. But it can hardly be to a greater *mere extent of surface above ground* that the property which the Leguminous plants possess of acquiring a greater amount of Nitrogen than the Graminaceous ones, over a given area of land, and under otherwise equal circumstances, is to be attributed.

A bean and a wheat crop may yield equal amounts of dry matter per acre, whilst the bean produce would contain from two to three times as much Nitrogen as the wheat. Nevertheless some attempts at approximate measurement have indicated that the wheat-plant offers a greater external superficies in relation to a given weight of dry substance, than does the bean. The wheat-plant would, of course, show a still higher relation of superficies to a given amount of Nitrogen fixed. If, therefore, the larger amount of Nitrogen yielded per acre by a bean than by a wheat crop be due to a larger assumption of it directly from atmospheric sources in some form, it is obvious that the result must be due to *character*, and *function*, and not to mere extent of surface above ground. In connexion with this point it may be observed, more particularly with reference to the crops that are grown for their ripened seed, that the Leguminous ones generally maintain their green and succulent surface in relation to a more extended period of the season of active growth and accumulation than do the Graminaceous ones.

(7) *Assimilation of free or uncombined Nitrogen by Plants.*—It has been seen, in the course of the foregoing brief review of the various sources of combined Nitrogen to plants, that those of them which have as yet been quantitatively estimated are inadequate to account for the amounts of Nitrogen obtained in the annual produce of a given area of land beyond that which may be attributable to the supplies by previous manuring. It must be admitted, too, that the sources of combined Nitrogen which have been alluded to as not yet even approximately estimated in a quantitative sense (if indeed they are all fully established qualitatively) offer many practical difficulties in the way of any such investigation of them as would afford results directly applicable to our present purpose. It appeared, therefore, that it would be desirable to settle the question, whether or not that vast storehouse of Nitrogen, the atmosphere, in which the vegetation which covers the Earth's surface is seen to live and flourish, be of any measurable avail to the growing plant, so far as its *free* or *uncombined Nitrogen* is concerned.

The settlement of this question (whether affirmatively or negatively) would at any rate indicate the degree of importance to be attached to the remaining open points of inquiry. Indeed, were it found that plants generally, or some of those we cultivate more than others, were able to fix Nitrogen from that presented to them in the free or uncombined form, we should, in this fact, have a clue to the explanation of much that is yet clouded in obscurity in connexion with the chemical phenomena of Agricultural production. We should establish for vegetation, the attribute of effecting chemical combinations with an element at once the most reluctant to associate itself with other bodies in obedience to laboratory processes and at the same time apt to rid itself of connexions once formed in the most violent manner—as the explosive character of many Nitrogen compounds forcibly illustrates. We should further be able, much more satisfactorily than we are at present, to account—by processes established to be going on under our own observation—for the actually large total amount of combined Nitrogen which we know to exist and to circulate, in land and water, in animal and vegetable life, and in the atmosphere.

But another and potent reason for investigating the relation of plants to the free or uncombined Nitrogen of the atmosphere is to be found in the fact, that the question has, of late years, been submitted to an immense amount of research by numerous experimenters, and from the results obtained very opposite conclusions have been arrived at. Thus, M. Boussingault concludes that plants do not assimilate the free or uncombined Nitrogen of the atmosphere. M. G. Ville maintains, on the contrary, that the assimilation of free Nitrogen does take place, and further, that, under favourable circumstances, a considerable proportion of the Nitrogen of a plant may be derived from this source. Others have experimented in connexion with the subject on a more limited scale ; and various explanations have been offered of the discrepant results and conclusions of M. Boussingault and M. G. Ville.

Before entering upon the discussion of our own experimental evidence in regard to the question of the assimilation of free or uncombined Nitrogen by plants, it will be desirable to pass in review the methods, results, and conclusions of M. Boussingault and M. G. Ville, and also of some other experimenters, who seem to have been led to take up the subject by a consideration of the contrary opinions arrived at by Boussingault and Ville.

Section IV.—Review of the researches of others, on the question of the assimilation of free nitrogen by plants, and on some allied points.

It has already been mentioned that, in 1837, Boussingault took up the question of the sources of the Nitrogen of Plants where De Saussure had left it more than thirty years before. De Saussure and his predecessors had sought to solve the question, among others, whether plants assimilated the free or uncombined Nitrogen of the atmosphere, by determining the changes undergone in the composition of limited volumes of air by the vegetation of plants within them. Boussingault pointed out that the methods which had been adopted were not sufficiently accurate for the determination of the point in question. The general plan instituted by himself, and adopted with more or less modification in most subsequent researches, was :—

To set seeds or plants, the amount of Nitrogen in which was estimated by the analysis of carefully chosen similar specimens.

To employ soils and water containing either no combined Nitrogen, or only known quantities of it.

To allow the access, either of free air (protecting the plants from rain and dust), of a current of air freed by washing from all combined Nitrogen, or of a fixed and limited quantity of air, too small to be of any avail so far as its compounds of Nitrogen were concerned. And finally—

To determine the amount of combined Nitrogen in the plants produced, and in the soil, pot, &c., and, so, to provide the means of estimating the gain or loss of Nitrogen during the course of the experiment.

A.—M. Boussingault's Experiments.

1. M. Boussingault's *experiments in* 1837 *and* 1838, *in which the plants were allowed free access of air, but were protected from rain and dust.*

In 1837 * Boussingault grew, in burnt soil, watered with distilled water, and with the access of free air, a pot of Trifolium for two months, and another for three months; also a pot of Wheat for two months, and another for three months.

The total Nitrogen in the seeds sown in the two experiments with *Trifolium*, amounted to 0·224 gramme. The Nitrogen in the produce, soil, pot, &c., amounted to 0·276 gramme. There was a gain, therefore, of 0·052 gramme of Nitrogen = nearly 20 per cent. of the total Nitrogen of the products. The development of vegetable matter, implying, of course, the assimilation of carbon, hydrogen, and oxygen, was, however, in a much greater proportion; the dry matter of the produce in the two experiments amounting to nearly three times that of the seed sown.

In the two experiments with *Wheat*, the total Nitrogen in the seed was estimated at 0·100 gramme. The Nitrogen in the products was exactly the same amount. In the case of the Wheat, there was, therefore, no gain of Nitrogen indicated. Nevertheless the *dry matter* of the produce amounted to nearly double that of the seed.

In 1838 †, Boussingault, in a similar manner, sowed *Peas* containing 0·046 gramme Nitrogen. The plants obtained, yielded flowers and ripe seed, and their dry matter was more than four times as much as that of the seed sown. The Nitrogen of the total products amounted to 0·101 gramme. Here again, therefore, the *Leguminous* plants seemed to gain Nitrogen from some undetermined source.

Boussingault made experiments in the same year (1838), with Trifolium, and with Oats. In these cases, he commenced with carefully selected plants instead of with seeds. The *Trifolium* nearly trebled its total vegetable matter during growth; and it gained 0·023 gramme of Nitrogen out of 0·56 gramme in the total products. The *Oat*, on the other hand, indicated only 0·053 gramme Nitrogen in the total products, whilst it was estimated that there was 0·059 gramme contained in the plants taken for the experiment. The total vegetable matter was, however, doubled.

The substance of M. Boussingault's conclusions from the above experimental results, may be stated as follows:—That under several conditions, certain plants seem adapted to take up the Nitrogen in the atmosphere; but that it was still a question, under what circumstances, and in what state, the Nitrogen was fixed in the plants. He submitted —that the Nitrogen might enter directly into the organism of the plant, provided its green parts were adapted to fix it; that it might be conveyed into the plant in the aërated water taken up by its roots; that, as some physicists suppose, there may exist in the atmosphere an infinitely small amount of ammoniacal vapour. He further suggested that the gain of Nitrogen beyond that supplied in manure, which he had observed in agricultural production on the large scale, and which he thought evidently

* Ann. de Chim. et de Phys. sér. 2. tome lxvii. 1838. † Ibid. tome lxix.

came from the atmosphere, might be partly due to Nitrate of Ammonia produced by electrical action and brought down by rain.

2. M. Boussingault's *experiments in* 1851, 1852, *and* 1853, *in which the plants were confined in limited volumes of air**.

Boussingault resumed the subject of the sources of the Nitrogen of vegetation in 1851. His object was, apparently, to settle more definitely, whether plants assimilated Nitrogen from any other source than the combined forms of it.

In his experiments in 1851 and 1852, Boussingault confined his experimental plants under a glass shade of about 35 litres capacity, which shut off the free access of external air by resting in a lute of sulphuric acid. Tubes passed under the shade for the supply of carbonic acid, and water, as they might be needed. Pumice-stone, coarsely powdered, washed, ignited, and cooled over sulphuric acid, served as soil. To this, at the commencement, some of the ash from farm-yard manure, and also from seed of the kind to be sown, was added.

In 1851, a Haricot was grown under these conditions, the seed of which, when sown, was estimated to contain 0·0349 gramme of Nitrogen. After two months of growth, flowers being formed, the dry substance of the plant was more than double that of the seed sown; and the total products yielded only 0·0340 gramme of Nitrogen. There was, therefore, apparently a slight loss of Nitrogen, which amounted, however, to less than a milligramme. In 1852, two Haricots, sown respectively in separate pots, contained, together, 0·0455 gramme Nitrogen. They were each allowed to grow for three months, during which time the dry substance was nearly doubled; and in one instance open flowers were formed. The products of both experiments taken together yielded to analysis only 0·0415 gramme of Nitrogen. There was an apparent loss, therefore, in the two experiments, of 4 milligrammes of Nitrogen. It is seen, then, that in these new experiments with *Leguminous* plants, in which the free circulation of atmospheric air was not permitted, there was not the apparent gain of Nitrogen that had been met with in Boussingault's early experiments (in 1837 and 1838), in which free access of air into the enclosing apparatus was allowed.

In 1851, ten seeds of Oats, and in 1852 four, were experimented upon in a similar manner. In both cases there was an apparent very slight loss of Nitrogen. In the first case the Oats vegetated for two months, and in the second for $2\frac{1}{2}$ months; and in the latter, the plant arrived at the point of shooting forth the ear.

In 1853, the apparatus adopted by Boussingault consisted of a large globe, or carboy, of white glass, having a capacity of 70 or 80 litres. At the bottom of this vessel, a matrix of pumice-stone (or burnt brick) and ashes, prepared as in the last series, was placed to serve as soil. This was watered with distilled water, and then the seeds were sown. The neck of the vessel was then closed with a cork, through a perforation in which, a flask of carbonic acid was inverted, whose aperture, opening into the globe, was

* Ann. de Chim. et de Phys. sér. 3. tome xli. 1854.

somewhat contracted. Finally, access of air from without was excluded by bandages of caoutchouc, which were so secured as to render the whole apparatus air-tight.

In such an apparatus, Boussingault made five separate experiments with White Lupins. In all he sowed thirteen seeds, which were estimated to contain, together, 0·2710 gramme of Nitrogen. The experiments extended over periods varying from six to eight weeks. In one instance, burnt brick instead of pumice-stone was used as the soil; and in this case, as well as in one where pumice was used, bone-phosphate as well as ashes were added as manure. The dry matter of the produce was about three times as much as was contained in the thirteen seeds sown. The Nitrogen in the total products of the five experiments amounted to 0·2669 gramme. There was, therefore, a loss, in the five experiments taken together, of about 4 milligrammes of Nitrogen. In two of the cases there was a slight gain of Nitrogen, but in neither instance did it amount to 1 milligramme.

In a similar apparatus, two experiments were made with Dwarf Haricots, a single seed only being sown in each case. One of the experiments extended over two months, and the other over two and a half months. In both instances flowers were formed, and in one of them seed. The dry matter of the produce was three to four times as much as that of the seed sown. Taking the two experiments together, the Nitrogen contained in the seed was estimated at 0·0652 gramme; and that found in the products amounted to 0·0637 gramme. There was a loss, therefore, of $1\frac{1}{2}$ milligramme of Nitrogen.

There was, then, in this third series of experiments with *Leguminous* plants, again rather a loss than a gain of Nitrogen,—the supplies of it in this case being confined to the combined Nitrogen contained in the seeds sown, and to the free or uncombined Nitrogen in the fixed and limited volume of air within the apparatus.

Still in the same apparatus, Boussingault sowed Garden Cress. Thirteen seeds were sown, all of which germinated, but three plants only survived. The growth of these extended over three and a half months; and flowers and seed were produced. The Nitrogen in the products amounted to precisely as much as was estimated to be contained in the thirteen seeds sown.

The last experiment in this closed globular apparatus was as follows: Two White Lupins were sown to grow; and eight others were applied as manure, after treatment with boiling water to destroy their powers of germination. The experiment continued for a period of between four and five months. The dry matter of the produce was nearly twice as much as would be contained in the ten seeds involved in the experiment. The whole ten seeds were estimated to contain 0·1827 gramme of Nitrogen; whilst the total products yielded only 0·1697 gramme. The loss of Nitrogen was here, therefore, 13 milligrammes; or about one-fourteenth of the whole amount involved in the experiment. Boussingault considered that the loss was probably due to free Nitrogen being given off in the process of decomposition of the organic matter employed as manure.

In order to ascertain whether the limitation of growth in the foregoing experiments was due to the limitation in the amount of air, or to a deficiency of available Nitrogen in the matters used as soil, Boussingault sowed Cress in a good soil, placed the vessel

in a limited atmosphere, and supplied carbonic acid. The result was, that the plants thus grown, in a limited atmosphere, but in a good soil, were even more luxuriant than a parallel set, grown in a similar soil, in the open air. In both cases a large quantity of seed was produced.

3. M. Boussingault's *experiments in* 1854, *with a current of washed air* *.

In this series of experiments, Boussingault supplied his plants with a *current* of air, previously washed by passing first through vessels containing pumice-stone saturated with sulphuric acid, and then through water. He also supplied carbonic acid from bicarbonate of soda acted upon by sulphuric acid,—the gas evolved being passed first over chalk, then through a solution of carbonate of soda, and lastly over pumice-stone saturated with a solution of carbonate of soda. The enclosing apparatus consisted of a metal-framed glass case of 124 litres capacity, which was cemented down upon a polished iron plate, upon which the experimental pots were placed. Across one side of the case was a metallic joint-bar, in which were apertures for the insertion of tubes for the admission of the washed air, and for the supply of water and carbonic acid. On the opposite side was a similar joint-bar, to an aperture in which, a tube was attached connecting the case with an aspirator of 500 litres capacity. By this apparatus, therefore, the plants could be supplied with a current of air freed from ammonia, with water, and with carbonic acid, at pleasure. During the experiment, the atmosphere in the Case generally contained from 2 to 3 per cent. of carbonic acid. Lastly, by means of one of the apertures any withered leaves were removed as they fell from the plants ; and they were then dried and preserved for analysis with the remainder of the products.

One of the experiments made in this apparatus was with a single Lupin, which was allowed to grow for two and a half months. The dry matter of the produce was more than six times that of the seed. The Lupin sown was estimated to contain 0·0196 gramme of Nitrogen. The Nitrogen found in the products amounted to 0·0187 gramme. There was a loss, therefore, of nine-tenths of a milligramme of nitrogen.

Four experiments were made with Dwarf Haricots, in three of which single seeds, and in the fourth two seeds, were sown. One experiment lasted over two and a half months, and the plant flowered ; one over three months, and the plant seeded ; one over three and a half months, in which case also the plant seeded ; and another over three and a quarter months. The dry substance of the produced plants was from three to four times as much as that of the seed sown. The total Nitrogen in the five seeds employed in the four experiments was estimated at 0·1672 ; the Nitrogen found in the total products amounted to 0·1661 gramme. There was therefore, upon the whole, a loss of 0·0011 gramme of Nitrogen. In two of the experiments there was a loss of 1 milligramme each of Nitrogen ; and in the other two a gain, amounting to less than 1 milligramme in each case.

In the next experiment, one Lupin seed was sown to grow, and another was steeped in hot water and applied as manure. The dry matter of the produce from the one seed amounted to nearly three times that of the two seeds employed in the experiment. The

* Ann. de Chim. et de Phys. sér. 3. tome xliii. 1855.

total Nitrogen in the two seeds was estimated at 0·0355 gramme. That found in the products was 0·0334 gramme. There was a loss, therefore, of 0·0021 gramme Nitrogen.

Lastly, forty-two seeds of Cress were sown, twelve of which served as manure. Many of the plants seeded. The dry matter of the produce was more than five times that of the seed. The Nitrogen in the forty-two seeds was estimated at 0·0046 gramme. That found in the products amounted to 0·0052 gramme. There was a gain, therefore, of 0·0006 gramme, or little more than half a milligramme of Nitrogen.

The whole of these experiments in 1854, in which a current of air was supplied to the plants, taken together, indicated a slight loss of Nitrogen. This was the case, notwithstanding that all the plants, excepting the Cress, were of the *Leguminous* family.

4. M. Boussingault's *experiments in* 1851, 1852, 1853, *and* 1854, *in which the Plants were allowed free access of air, but were protected from rain and dust**.

Contemporaneously with the several series of experiments above described, Boussingault grew plants simply covered with a case, in such a manner as to exclude any material amount of dust, but so as to allow of the free access of the external air.

Single Haricots were grown in the manner here described, in the seasons of 1851, 1852, 1853, and 1854, respectively. All four plants flowered; one podded; and one seeded. The Nitrogen in the seed of the four experiments amounted to 0·1173 gramme. That found in the vegetable produce, soil, &c., was 0·1238 gramme. There was a total gain of Nitrogen, therefore, under these circumstances, of 0·0065 gramme. In one case there was an apparent loss of Nitrogen of a little more than 2 milligrammes; in the three others the gain was about equal. The dry matter in the produce amounted to from three to four times as much as that in the seeds sown.

In the seasons of 1853 and 1854, three experiments of the same kind were made with White Lupins. The dry matter of the produce was from three to four or more times as much as that in the seed. The Nitrogen in the seed of the three experiments taken together amounted to 0·0780 gramme. That in the total products was 0·0873 gramme. Here again, therefore, there was a gain of Nitrogen—amounting in this case, in all, to between 9 and 10 milligrammes.

Under similar conditions, Oats were grown in 1852 which yielded seed. The Nitrogen sown was 0·0031 gramme. That in the products was 0·0041 gramme. There was a gain, therefore, of 1 milligramme of Nitrogen.

In like manner, five seeds of Wheat were sown in 1853. The dry matter of the produce was more than three times that of the seed. The Nitrogen in the seed was estimated at 0·0064 gramme. That in the products was 0·0075 gramme. The gain was, therefore, 0·0011 gramme.

Lastly, 210 seeds of Cress were sown in 1854. Many of the plants seeded; and there was, of course, a considerable gain of dry matter. The Nitrogen in the seed was 0·0259 gramme. That in the products amounted to 0·0272 gramme. There was a gain, therefore, of 0·0013 gramme.

* Ann. de Chim. et de Phys. sér. 3. tome xliii. 1855.

Taking all these experiments together, in which the plants were shaded from rain and dust, but still allowed free access of air, the total gain of Nitrogen was 0·0192 gramme upon 0·2307 gramme supplied in the seed sown. There was a gain of Nitrogen, therefore, equal to about one-twelfth of that sown in the seed. Boussingault considered that part of the gain was due to organic corpuscles, and part to the ammonia in the atmosphere. He also considered that, bearing in mind the circumstances of the experiment, the gain was not sufficiently great to justify the conclusion that there had been any assimilation of the free or uncombined Nitrogen of the air.

5. M. Boussingault's *collateral experiments to control and explain his results**.

In order to ascertain the amount of Nitrogen that might be introduced into the materials under experiment when the matter used as soil, &c. was not excluded from the air whilst cooling after ignition, or when free access of air was allowed during the whole period of vegetation, Boussingault instituted the following experiments.

Sand, powdered brick, powdered bone-ash, and wood-charcoal were each exposed to the air for two or three days after being ignited, and then the Nitrogen determined in them. The result was that, after this exposure, a kilogramme of sand gave 0·5 milligramme, a kilogramme of powdered brick 0·5 milligramme, a kilogramme of powdered bone-ash 0·84 milligramme, and a kilogramme of wood-charcoal 2·9 milligrammes of ammonia.

In order to test the influence of the organic corpuscles of the atmosphere, a pot of burnt sand, with ashes, the whole moistened with water, was so arranged under a shade as nevertheless to allow free access of air, and it was so exposed for two and a half months. At the end of this period small spots of cryptogamic vegetation were visible on the surface of the sand ; but the whole yielded only 0·74 milligramme of Nitrogen.

Again, Boussingault found that unless the ashes used as manure were burnt until nearly all apparent traces of carbon were destroyed, they were liable to retain more or less and sometimes material amounts of Nitrogen. In some imperfectly burnt ashes cyanides, and in some, ferrocyanides were found; in others the Nitrogen seemed to exist in neither of these conditions.

With regard to the much larger gain of Nitrogen indicated in his early experiments in free air (1837 and 1838) than in those made more recently, Boussingault remarks that the result may be partly due to the comparatively defective methods of analysis at the early date, and partly also to the distilled water used for watering the plants containing some ammonia. For, at the time of his first experiments, he was not aware of the fact, since learned in his analyses of rain and other waters, that water distilled from that which contained minute quantities of ammonia did not come over free from it until about two-fifths of the whole had been drawn off.

It will be observed that, in most of the experiments of Boussingault thus far passed in review, he limited the supply of Nitrogen to the plants to that contained in the seed sown, and to that which they could obtain from the atmosphere, either washed or un-

* Ann. de Chim. et de Phys. sér. 3. tome xliii. 1855.

washed, in which they grew. In no case among those experiments in which the modern refinements of analysis were had recourse to did he find, either with Leguminous or with other plants, such a gain of Nitrogen beyond that supplied in the seed, as could lead to the conclusion that the free or uncombined Nitrogen of the atmosphere had been assimilated. In many of the instances the plants yielded not only flowers but seed; and hence it might be concluded that the conditions provided were adequate for the performance, by the plant, of the complete course of its natural functions of growth. Still it might be objected that the vigour of growth was somewhat limited, and that, under these circumstances, the plant might well refuse to perform the, perhaps, difficult office of assimilating a very refractory elementary body. In a few instances, seeds whose germinating power had been destroyed were supplied as manure. In these cases the amount of Nitrogen assimilated by the plants was much greater than that contained in the living seed sown; and the luxuriance of growth was consequently comparatively great. Nevertheless, instead of a gain, there was generally a loss in the total amount of combined Nitrogen, which was considered to be due to the evolution of free Nitrogen by the decomposing manurial matter. To get increased vigour of growth—to avoid, if possible, a loss of Nitrogen such as is above supposed—and, at the same time, to determine whether or not the Nitrogen of Nitrates was really assimilable by plants—Boussingault has latterly made some experiments in which Nitrates were employed as manure, a brief notice of the results of which should be here given.

6. M. Boussingault's *experiments in which he supplied combined Nitrogen in the form of Nitrate of Potash, or Soda**.

In 1855 Boussingault made one experiment with Helianthus in which he supplied no nitrate to the soil, and another in which a small known quantity of Nitrate of Potash was employed. In a third experiment Cress was grown in a manured soil, in a fourth in a soil destitute of combined Nitrogen, and in a fifth in a soil to which a known quantity of Nitrate of Soda was added. In the case of the manured soil, and in the two cases where Nitrate was employed, there was a very considerable increase in the assimilation of carbon; and there was also much more Nitrogen assimilated than was supplied in the seeds sown. The increased assimilation of Nitrogen where Nitrate was used, did not, however, exceed that supplied in the manure. Boussingault concluded that the gain of Nitrogen was to be attributed to the Nitrogen of the Nitrate.

Lastly in regard to Boussingault's experiments: In 1858† he resumed the question of the action of Nitrates upon vegetation. He grew two separate pots of Helianthus, two seeds being sown in each pot. The soils were composed of sand and quartz well washed from saline matter and ignited. To one pot Nitrate of Potash containing 0·0111 gramme of Nitrogen, and to the other Nitrate containing 0·0222 gramme Nitrogen, was added. In the first case, he did not get back, in the plant, soil, and pot, the Nitrogen of the seed and Nitrate by 0·0014 gramme. In the second experiment the loss of Nitrogen amounted to just 1 milligramme. Boussingault found, however, that there remained

* Ann. de Chim. et de Phys. sér. 3. tome xlvi. 1856. † Compt Rend. tome xlvii. 1858.

in the soil an amount of carbonate of potash very nearly corresponding in potash to the amount of nitrate of potash which would represent the observed loss of Nitrogen. He concluded that nitrate had been decomposed in the soil, by the organic matter of the débris of the seeds and of the roots, and that Nitrogen had been evolved. If we clearly understand this explanation of the loss of Nitrogen of the nitrate, we would suggest that it would seem to require for its validity that the plant should have assimilated potash from the nitrate exactly corresponding in amount to the Nitrogen it fixed from the same source.

From the results of these experiments with nitrate, Boussingault drew the following conclusions :—

1. That there was no assimilation of free Nitrogen.
2. That there was a loss of supplied Nitrogen, either from the soil, or by the plant.
3. That, in the two cases, the amount of carbon assimilated bore a close relation to that of the Nitrogen taken up by the plant.

It is seen, then, that the results of the laborious investigations of Boussingault, extending at intervals over a period of more than twenty years, have led him to conclude that, neither *Leguminous* plants, nor the others experimented upon, were able, either when their supplies of combined Nitrogen were limited to that contained in the seed sown, or when their vigour of growth was stimulated by artificial supplies of combined Nitrogen, to assimilate the free or uncombined Nitrogen of the atmosphere.

B.—M. G. Ville's Experiments*.

1. M. G. Ville's *determinations of the Ammonia in the atmosphere.*

M. G. Ville, of Paris, commenced his investigations, on the subject of the assimilation of Nitrogen by plants, in 1849. He first sought to determine the proportion of Ammonia in the atmosphere. To this end, he aspired known quantities of air through acid, and determined the amount of ammonia absorbed. He operated upon very much larger volumes than previous experimenters had done. His results show, moreover, a much smaller proportion of ammonia in the air than those of others.

The air of Paris, during part of 1849 and part of 1850, gave a mean of only 0·0237 part by weight of ammonia, to 1,000,000 parts by weight, of air ; and that of the suburbs of Paris, during some period of 1852, gave 0·0211 part of ammonia, to 1,000,000 parts of air.

2. M. G. Ville's *general plan of experimenting on the question of the assimilation of Nitrogen by plants.*

M. G. Ville employed specially-made porous flower-pots, and used, as soil, washed

* Recherches Expérimentales sur la Végétation, par M. Georges Ville. Paris, 1853.

and ignited sand, sand and brick, or sand and charcoal, with the addition of the ash of the plant to be grown. He planted seeds or plants, the composition of which was estimated by the analysis of parallel specimens. Several pots were for the most part enclosed in an iron-framed glazed case of 150 litres (or more) capacity, through which a current of air equal in amount to several times the volume of the vessel was aspired daily. Carbonic acid and distilled water were supplied as needed. In some cases the air admitted into the apparatus was only previously freed from dust; and then the amount of atmospheric ammonia that would be brought in was calculated according to the determination of the proportion of ammonia in the air, above alluded to. In other cases the aspired air was previously freed from ammonia by washing. In some experiments, ammoniacal gas was passed, in known quantities, into the air of the apparatus. Lastly, others were made, in which combined Nitrogen was added to the soil in the form of nitrate, or of ammonia salts; and in these cases the plants were allowed to grow in free air, only shaded from rain and dust.

3. M. G. Ville's *experiments in* 1849 *and* 1850, *in which the plants were supplied with a current of unwashed air.*

In 1849, sand was used as soil; three pots of plants were grown for two months; namely, one of Cress, one of large Lupins, and one of small Lupins. The air admitted into the apparatus was not previously deprived of its natural ammonia. The dry substance of the produced Cress plants amounted to more than sixteen times that of the seed sown. The Nitrogen in the Cress seeds amounted to 0·026 gramme; that in the products to 0·147 gramme. The Nitrogen in the products was, therefore, between five and six times as much as that in the seed; and the actual gain of it amounted to 0·121 gramme. In the case of the large Lupins, the dry matter of the produce was about $3\frac{1}{2}$ times as great as that of the seeds sown; but there was neither gain nor loss of Nitrogen. The small Lupins gave $2\frac{1}{2}$ times as much dry substance in the produce as was supplied in the seeds; and of the Nitrogen of the seeds sown, there was an apparent loss of rather more than one-fourth, during the experiment.

The total gain of combined Nitrogen in the apparatus, taking the three experiments together, was 0·103 gramme. The Nitrogen in the ammonia of the current of unwashed air, was, however, estimated at only 0·001 gramme. M. G. Ville concluded, therefore, that the Cress had appropriated a considerable quantity of the free or uncombined Nitrogen of the atmosphere.

The plants experimented upon in 1850, were Colza, Wheat, Rye, and Maize. In the case of the Colza, the experiment commenced with young plants, but in the other cases with seed. The four pots were placed in an apparatus similar to that used before, and the conditions supplied were also the same as in 1849.

The dry matter of the produced Colza plants amounted to between forty and fifty times as much as that of the young plants when taken for experiment. The Nitrogen was also increased more than forty-fold. The dry matter of the Wheat plants was about

four times that of the seed sown ; and the Nitrogen in the total products was nearly double that in the seed. The dry substance of the produced Rye plants was five times, and their Nitrogen nearly three times that of the seed. In the experiment with Maize, the dry matter increased only about three times, but the Nitrogen nearly $4\frac{1}{2}$ times.

The actual gain of Nitrogen in the total products of the four experiments, was 1·1803 gramme. The whole admitted in the form of atmospheric ammonia was estimated at 0·0017 gramme, or less than 2 milligrammes. M. Ville remarks, moreover, that an examination of the distilled water before being used to water the plants, and of the water afterwards removed from the apparatus, showed more ammonia in the latter than in the former. The conclusion from this second series of experiments also was, therefore, that a considerable quantity of free or uncombined Nitrogen had been assimilated.

4. M. G. Ville's *experiments in* 1851 *and* 1852, *in which the plants were supplied with a current of air washed free from ammonia.*

In 1851, one pot of Sun-flower, from seed, and two pots of Tobacco, starting from plants transplanted from good soil, were grown together, under circumstances similar to those of the preceding experiments, with the exception that now the air was deprived of its ammonia by passing over pumice-stone saturated with sulphuric acid, and also through a solution of bicarbonate of soda, previous to entering the apparatus.

The Sun-flowers gave 95 rudimentary grains ; but the Tobaccos did not flower. However, taking the three experiments together, the dry matter increased nearly 200-fold, and the Nitrogen increased nearly 40-fold, during a period of growth of three months. The total gain of Nitrogen in the apparatus was 0·481 gramme.

In 1852, the conditions of the apparatus were the same as in 1851. The selection of plants was as follows :—One pot of Autumn Colzas, starting with young plants ; one of Spring Wheat, from seed ; one of Sun-flower, from seed ; and two of Summer Colzas, from plants.

In every case the dry matter of the produce was many times that of the young plants or seed. In the case of the Sun-flower, it was more than 100 times that of the seed. In each experiment, there was of Nitrogen, also, much more at the conclusion, than at the commencement. In the experiment with Autumn Colzas there were 4·7 times, in that with Spring Wheat 2·2 times, in that with Sun-flower 25·5 times, in one with Summer Colza 3·4 times, and in the other with Summer Colza 6·7 times as much Nitrogen in the total products as in the original plants or seeds. The total amount of Nitrogen gained in the five experiments, was 1·624 gramme, which was 5·3 times as much as was contained in the total original plants and seeds.

To show the degree of luxuriance of growth of the different descriptions of plant, it may be mentioned that the Winter Colzas flowered, but gave no seed ; the Sun-flower gave 412 rudimentary grains ; and the Wheat seeded completely, giving 47 grains. The Summer Colzas had little tendency to go to seed, but developed a great deal of leaf; and hence it was, it was supposed, that they gained large actual amounts of Nitrogen.

5. M. G. Ville's *experiments in which known quantities of Ammonia were admitted into the atmosphere of the enclosing apparatus.*

In each of the three seasons 1850, 1851, and 1852, M. G. Ville had a duplicate apparatus, enclosing, in each case, similar plants to those in the other, but with this difference in the conditions—that ammonia was supplied to the atmosphere of the second apparatus. As might be expected, the increase, both in dry substance, and in Nitrogen, was much the greater, in relation to the amounts of them contained in the seed or young plants, when ammonia was thus employed. In no case, however, did the plants take up Nitrogen equal in amount, much less exceeding, the whole of that supplied to the air in the combined form, as ammonia. The results have not, therefore, so direct a bearing on the question of the assimilation of free or *un*combined Nitrogen, as to require that we should quote them in any detail. Their chief interest was in showing the influence of ammoniacal supply, not only upon the vigour and luxuriance of growth generally, but upon the order, or course of development, of the plants, according to the stage of growth at which the application was made.

6. *Comparison of* M. G. Ville's *results with those of* M. Boussingault *up to* 1853 *inclusive.*

It will be remembered that, up to 1853 inclusive, M. Boussingault's experimental plants had been grown either in *free air*—in which case they had fixed, from some source, slightly larger amounts of Nitrogen than were contained in the seed,—or in fixed and *limited volumes* of air (carbonic acid being added), in which cases no gain of Nitrogen was observed. The gain of Nitrogen in the free air was, moreover, considered to be too small to indicate, under all the circumstances, any assimilation of free or uncombined Nitrogen. On the other hand, M. G. Ville's experiments up to the same period had indicated an enormous gain of Nitrogen. The Nitrogen in the products, indeed, sometimes amounted to more than forty times that contained in the seed.

Results so strikingly contradictory could hardly fail to excite great attention and interest among Chemists and Vegetable Physiologists. But M. Ville's plants had been grown in a *constant current of renewed air*, and not in only a *fixed and limited volume* of it. This fact, and some other points, were alleged to account for the difference in result. At any rate, on the one hand, M. Boussingault commenced in 1854, to experiment with a *current of air*; whilst, on the other, a Commission, composed of Members of the Academy of Sciences of France, was appointed to superintend the conduct of a new set of experiments by M. G. Ville. It has already been shown, that M. Boussingault's new experiments in which a *current of air* was employed, did not indicate any assimilation of free or uncombined Nitrogen, any more than did those in which the plants had grown in limited volumes of air only.

7. M. G. Ville's *experiments conducted under the superintendence of a Commission comprising* MM. Dumas, Regnault, Payen, Decaisne, Peligot, *and* Chevreul.

These experiments were conducted at the Muséum d'Histoire Naturelle, Jardin des Plantes, Paris, in the autumn of 1854. M. Cloez was appointed to assist M. Ville; and M. Chevreul reported on behalf of the Commission, in 1855*.

In an apparatus similar to that employed in the experiments of M. Ville which have been already described, three pots of Cress were placed. The soil consisted of ignited brick and sand, to which was added some of the ash of the plant. Carbonic acid was supplied artificially; and the plants were watered with distilled water. The Cress in one of the pots did not thrive well; and, in this case, analysis showed a loss of 2 milligrammes of Nitrogen. In one of the other cases, there was a gain of 0·0492 gramme of Nitrogen, upon 0·0038 gramme supplied in the seed; and in the other, there was a gain of 0·0071 gramme of Nitrogen, upon 0·0039 gramme contained in the seed.

At the suggestion of one of the members of the Commission, a smaller vessel was also attached to the aspirator, in which one pot sown with Cress was placed. The soil being duly watered with distilled water, the apparatus was then closed, and not opened (as the other frequently was) until the conclusion of the experiment. In this case also, there was a considerable gain of Nitrogen indicated, namely, 0·0287 gramme gain, upon 0·0063 gramme in the seed.

Unfortunately, an element of uncertainty attached to the evidence afforded by these experiments made under the superintendence of the Commission, which is very much to be regretted. A quantity of distilled water taken from the same bulk as that used for watering the experimental plants was saved for analysis. The examination of this water devolved on M. Cloez; who, unfortunately, was called away for some days, during the evaporation of the water with oxalic acid, with a view to the after-determination of any ammonia it might contain. M. Peligot determined the ammonia in the acid residue of the evaporation of this water, as well as in that of the water removed from the cases, after it had served in the experiments. The result was, that there was indicated such an excess of ammonia in the water before being used, over that in the residual water after removal from the larger case, as more than covered the increase in the Nitrogen of the plants over that in the seeds sown. M. Cloez found, however, that in his absence, the evaporation of the water had been conducted by the side of ammoniacal emanations from other processes. But when new portions of the original water were evaporated with proper precautions, less ammonia was indicated in it than in the water at the close of the experiment; and then, also, a gain of Nitrogen by the plants in the larger apparatus was indicated.

At any rate, however, the result with the single pot, in the small apparatus, showed a considerable gain of Nitrogen, even supposing the first analysis of the supplied water to be correct.

* Compt. Rend. 1855

From the result of the whole inquiry, the Commission announced the following conclusion :—

That the experiment made at the Muséum d'Histoire Naturelle by M. VILLE, *is consistent with the conclusions which he has drawn from his previous labours.*

8. M. G. VILLE'S *experiments in which the plants were exposed to free air, and Nitrates or Ammonia salts were employed as manure**.

In 1855 and 1856, M. G. VILLE conducted a series of experiments with the double object, of investigating the action of nitrates upon vegetation, and of still further examining into the capability of plants to assimilate the free or uncombined Nitrogen of the atmosphere. The whole of the experiments now in question were made in free air, the plants being only shaded from rain ; that is to say, without any enclosing apparatus, or artificial current of air and supply of carbonic acid. The soils consisted of calcined sand ; ashes of plants such as those to be grown were added ; and distilled water was used for watering. Colza and Wheat were the plants experimented upon. Lastly, the special conditions of the experiments were, that nitrate of potash in smaller or in larger quantity, or nitrate of potash and different ammonia salts, in equivalent quantities so far as their Nitrogen was concerned, were employed.

To the prosecution of this series of experiments, an exact method of estimating minute quantities of nitric acid was essential. M. VILLE succeeded in devising such a method, which was very favourably reported upon by M. PELOUZE, on behalf of a Commission composed of MM. BALARD, PELIGOT, and PELOUZE.

In 1855, two pots, and in 1856 one pot, of Colzas were grown, to each of which 0·5 gramme of nitrate of potash was supplied as manure. By examination of the soil, the point was ascertained when the whole of the nitrate had been drawn from it by the plants. The experiment was then stopped ; and analysis showed that the total produce contained almost identically the amount of Nitrogen supplied in the seed and in the nitrate. The dry vegetable substance was, however, increased about 200-fold.

Again in 1855, two pots of Colzas were sown, to each of which 1 gramme instead of 0·5 gramme of nitrate was added ; and in 1856 two more, with the same quantity. In each of these cases, the produce (which in dry matter was several hundred times that of the seed) contained considerably more nitrogen than had been supplied in the seed and in the nitrate. M. G. VILLE's conclusions were, that the plants had taken up the nitrate and assimilated its Nitrogen, and that when by the larger supply of nitrate the growth had been extended, the free Nitrogen of the atmosphere was also assimilated.

In 1855 an experiment was made with Wheat manured with 1·72 gramme of nitrate of potash. The plants were allowed to mature, and they gave 84 grains. There was more Nitrogen in the vegetable produce alone, than in the seed and nitrate, and very much more in the total products, taking into account the residual Nitrogen in the soil. In 1856, two pots of Wheat were sown, to each of which 1·765 gramme of nitrate was added. The plants of one pot were taken up at the time of flowering, and they contained

* Recherches Expérimentales sur la Végétation, 1857.

almost identically the same amount of Nitrogen as was provided in the seed and nitrate. Those in the other pot were allowed to go to seed, and 119 grains were formed. In this case, again, the Nitrogen in the produce was much more than had been supplied, and very much more when the residual Nitrogen in the soil and pot was taken into the calculation. Lastly, on this head, two pots of Wheat (also in 1856) were sown without nitrate, and two with 0·792 gramme of nitrate to each. There was a considerable gain of Nitrogen in each of the four cases. The actual amount of gain was greater in the cases where the nitrate was employed; but the proportion gained, to that supplied, was greater where no nitrate was used.

To show the comparative efficacy of Nitrogen supplied in different conditions of combination, the following experiments were made during the season of 1856.

Two pots of Colzas received, each 0·5 gramme of nitrate of potash; and two other pots of Colzas received each an amount of sal-ammoniac equivalent in Nitrogen to the 0·5 gramme of nitrate. The two experiments with Nitrate gave equal amounts of Nitrogen in the produce; and the two with sal-ammoniac, also equal amounts. But the two with nitrate gave more than $1\frac{1}{2}$ time as much Nitrogen in the produce, as the two with sal-ammoniac. In two other experiments, double the quantity of nitrate and sal-ammoniac, respectively, was employed, and the growth was allowed to extend over a longer period. The amount of Nitrogen in the produce was, in both these cases, very much greater in proportion to the amount supplied, than in the preceding experiments where the smaller amounts of nitrate and sal-ammoniac were used. It was, moreover, more than three times as much where the nitrate, as where the sal-ammoniac was employed. There was, too, where the nitrate was used, a considerable amount of Nitrogen assimilated beyond that provided, in the combined form, in the seed and manure.

Experiments similar to the above were made with Wheat. Two pots, to each of which nitrate of potash was added, containing 0·110 gramme of Nitrogen, yielded, respectively, in produce, 0·218 and 0·224 gramme of Nitrogen. Two pots of Wheat, each manured with sal-ammoniac, containing also 0·110 gramme of Nitrogen, gave, respectively, in the produce, 0·161 and 0·124 gramme of Nitrogen. In the same way, nitrate of ammonia containing 0·110 gramme of Nitrogen gave 0·118 and 0·149 gramme, and phosphate of ammonia 0·116 and 0·150 gramme of Nitrogen in the matured Wheat plants.

In regard to the experiments of M. Ville referred to in this Division (8), he remarks, that the point at which the artificially supplied Nitrogen becomes exhausted is indicated by a lightening of the colour of the leaves, and that it is then that the plants begin to assimilate the uncombined Nitrogen of the atmosphere. To secure this assimilation, he considers that it is not only necessary that the supply of combined Nitrogen, and the vigour of growth, should reach beyond a certain limit, but that the artificial supply itself should, on the other hand, not exceed a certain limit. Further, the gain of Nitrogen in the experiments conducted on the plan now under consideration was so great, that, bearing in mind previously obtained results wherein the limit of the effect

of atmospheric ammonia had been ascertained, the influence of that source may, in the case of these new results, be entirely overlooked.

The fact that a given amount of Nitrogen in the form of combination of a nitrate was more efficacious than the same amount supplied in either of the ammoniacal salts experimented upon, was held to show that the nitrate was taken up by the plants as such, and was not previously transformed into ammonia.

M. Ville's experiments, as a whole, thus indicated that plants can take up Nitrogen in three forms—namely, as nitric acid, as ammonia, and as free Nitrogen. He enumerates the following conclusions :—

1. By means of nitre we may prove, without the aid of an enclosing apparatus, that plants absorb and assimilate the gaseous Nitrogen of the atmosphere.
2. Nitre acts by its Nitrogen. It is absorbed in the state of nitre.
3. In relation to the amount of Nitrogen, nitre is more active than ammonia-salts.

9. M. G. Ville's *collateral experiments to control or explain his results**.

M. Ville adduces evidence of yet another kind, in support of his view that plants assimilate the free Nitrogen of the air. Air was passed through an otherwise closed apparatus, in which was placed a vessel containing calcined sand, or calcined sand and decomposing organic matter. In no case was nitric acid formed. Nitrification, the result of the combination of the oxygen and nitrogen of the air within the porous soil, was not, therefore, the source of the Nitrogen gained by his experimental plants.

Experiments were made in which a given amount of organic matter (Lupins, Gelatine, &c.) was mixed with calcined sand, and exposed in an apparatus to a current of air, which carried the gaseous products into acid, to retain any ammonia that might be formed. The determination of the Nitrogen remaining in the matrix, and of the ammonia given off and absorbed by the acid, showed a loss of Nitrogen, which could only have passed away in the free gaseous form.

Other vessels of sand were prepared, to which similar known amounts of organic matter were added, and then seeds of Wheat were sown, the organic matter serving as manure. When the growth was stopped at a certain stage, almost exactly the same amount of Nitrogen was found in the Wheat plants and in the sand, &c., as was originally contained in the seeds sown and in the organic matter added. Assuming that the decomposition of the organic matter had taken the same course as in the other experiments—free Nitrogen being given off—it was obvious that a corresponding amount of free Nitrogen had been taken up by the plants. In other cases the growth of the Wheat was allowed to continue longer than in the experiments just alluded to; and then the total Nitrogen in the products not only equalled, but considerably exceeded, that in the seed sown and in the organic manure. In this instance, at least, it could not be said that the Nitrogen not received by the plant as ammonia had been taken up by it as nascent Nitrogen evolved in the decomposition.

* Recherches Expérimentales sur la Végétation, 1857.

The general conclusions from this part of the inquiry were as follow :—

1. Organic matters in decomposition lose a part of their Nitrogen as ammonia, and a part as Nitrogen gas.

2. Vegetation does not interfere with the progress of this decomposition.

3. Plants cultivated in a manured soil, give more Nitrogen in their produce than the manure yields as ammonia.

4. The excess of Nitrogen in the produce has been absorbed as free gaseous Nitrogen.

In regard to the explanation of the assimilation of free Nitrogen by plants, M. Ville calls attention to the fact, that nascent hydrogen is said to give ammonia, and nascent oxygen nitric acid, with free Nitrogen ; and he asks—Why should not the Nitrogen in the juices of the plant combine with the nascent carbon and oxygen in the leaves ? He further refers to the supposition of M. de Luca, that the Nitrogen of the air combines with the nascent oxygen given off from the leaves of plants, and forms nitric acid. Again, the juice of some plants (mushrooms) has been observed to ozonize the oxygen of the air ; is it not probable, then, that the Nitrogen dissolved in the juices will submit to the action of the ozonized oxygen with which it is mixed, when we bear in mind that the juices contain alkalies, and penetrate tissues the porosity of which exceeds that of spongy platinum, a body so apt to favour combinations ?

Summary Statement of the results and conclusions of M. Boussingault *and* M. G. Ville.

M. Boussingault, when, in his earlier investigations, he grew plants in free air, found only such indications of a gain of Nitrogen as, in his opinion, may be attributed to inaccuracies in the methods of experimenting and analysis at the early date, and to the combined influences of ammonia and organic corpuscles in the atmosphere ; and when, more recently, he grew plants only shaded in such a manner as still to allow the free access of air, the gain of Nitrogen observed was not more than he considered might be due to the influences last mentioned. When he grew plants, either in confined and limited volumes of air, or in a current of air washed free from ammonia and organic corpuscles, the results did not show any appreciable gain of Nitrogen. Lastly, when he supplied either decomposing organic matter, or nitrate, to increase the activity of growth, he did not find such an amount of combined Nitrogen in his products, as to lead him to conclude that there had been any assimilation by the plants of free or uncombined Nitrogen. In these cases, indeed, he generally found a loss of combined Nitrogen during the experiment, supposed to be due to the evolution of free Nitrogen in the decomposition of the matters used as manure.

The results of M. G. Ville, on the other hand, showed a very considerable gain of Nitrogen during growth, whether the plants were subjected to a current of unwashed air, or of ammonia-free air,—and also when the plants were grown in free air, and their activity of development increased by the use of nitrates, or other nitrogenous matters, as manure. This gain of Nitrogen he considers to be due to the assimilation of free or uncombined Nitrogen. It is remarkable, too, that the proportion of Nitrogen gained, to

that supplied in the combined form, was observed to be the largest in some of the cases where no nitrogenous manure was employed, and where the total amount of combined Nitrogen within the reach of the plants was confined to a few milligrammes only, contained in the original young plants or seeds that were planted. In some such instances, the amount of combined Nitrogen found in the products was about forty times as much as was supplied. In other cases, the assimilation of free Nitrogen only seemed to take place when the activity, and stage of growth, of the plants, had been forced beyond a certain point by the use of considerable amounts of nitrogenous manure.

Results and conclusions so astonishingly conflicting as those of M. Boussingault and M. G. Ville, have naturally incited others, either to investigate anew, or to seek, in the conditions provided in their experiments, for some explanation of the discordance. Before entering upon the consideration of our own experiments bearing upon the points in question, it will be desirable to add to the foregoing review a brief notice of the labours, or opinions, of these other experimenters or arbitrators.

C.—M. Mène's Experiments*.

In 1851, M. Mène made some experiments in reference to the assimilation of Nitrogen by plants. He seems to assume that Boussingault had concluded from his experiments that the free Nitrogen of the atmosphere was appropriated by plants; and he refers to the experiments of M. G. Ville as confirmatory of such a view. M. Mène made three sets of experiments in reference to this question.

1. He grew Wheat and Peas, respectively, in powdered glass as soil, allowing them contact with common air, and watering them with pure water. The Wheat increased in Nitrogen in amount equal to one-fourth of that contained in the seed sown; whilst its carbon, hydrogen, and oxygen were double those of the seed. The Pea-plants doubled the carbon, oxygen, and hydrogen of the seed sown, and their Nitrogen was threefold that of the seed.

2. Lentils, Peas, Haricots, Beans, Wheat, Rye, and Oats were grown in a sterile matrix under a bell-glass. They were respectively supplied with an atmosphere of known composition, and with acetate of ammonia in the soil. The plants increased in Nitrogen, and the ammonia in the soil diminished; but the free Nitrogen of the air was not perceptibly affected.

3. This series of experiments was in every way similar to the second, with the exception that the Nitrogen of the air was replaced by hydrogen. The plants flourished, and took up some of the acetate of ammonia.

M. Mène concludes that plants do not appropriate the free Nitrogen of the air.

D.—M. Roy's Views on the subject of the Assimilation of Nitrogen by Plants†.

M. Roy gave a paper on this subject in 1854. His supposition was that carbonate of ammonia constituted the chief source of Nitrogen to plants. Leguminous plants, he

* Compt. Rend. xxxii. † Ibid. xxxix.

considered, appropriated carbonate of ammonia from the atmosphere by their leaves. Graminaceous crops, on the other hand, he supposed, only took it up in solution by their spongioles. He further supposed that the free Nitrogen of the air was not appropriated by the leaves of plants, but that Nitrogen dissolved in water, and so taken up, by their roots, could be assimilated. He concluded that, in the case of M. Boussingault's plants grown in limited air, there would be but little passage of solution of Nitrogen by their roots, and evaporation of water from their leaves, and that, hence, the necessary conditions did not exist for the assimilation of free Nitrogen. M. Ville's rapid current of air would, on the other hand, cause a considerable amount of solution of Nitrogen to be drawn into the plants.

E.—The Experiments of MM. Cloez and Gratiolet.

In 1850, MM. Cloez and Gratiolet published the results of some experiments made with Water-plants. They found that, carbonic acid and air being both present, the plants gave off oxygen slowly, or very rapidly, according to the condition of the sunlight and the temperature. In water deprived of common air, but containing carbonic acid, the evolution of oxygen rapidly declined, Nitrogen was given off, and the plant contained less Nitrogen than a similar plant in water not deprived of its air. The evolution of Nitrogen diminished as the experiment proceeded. They considered that, in the vegetation of Water-plants, Nitrogen is given off from their nitrogenous constituents and that there must be restoration either from free or combined Nitrogen. And as their experiments showed that ammonia-salts were injurious to the plants, they concluded that they take up free Nitrogen dissolved in water.

In 1855* M. Cloez published the results of some experimental inquiries on nitrification, with a view to the question of the source of the Nitrogen of plants. He made twenty experiments, passing washed air through as many different combinations of porous, earthy, and alkaline matters. The experiments continued from September 1854 to April 1855, when, in some cases, efflorescence was observed. He found nitrates to be formed in notable quantity in calcined brick, or pumice, impregnated with alkaline or earthy carbonates; also, in uncalcined brick similarly impregnated. In chalk, marl, a mixture of kaolin and precipitated carbonate of lime, &c., only traces of nitrate were formed.

M. Cloez concluded that nitrates would be formed when a current of air was passed over porous bodies, alkalies being present. He considered, therefore, that the porosity of the pots and brick fragments, the alkalinity of the ashes, the moisture, and the current of air, in M. Ville's experiments with plants, provided the conditions for the formation of nitric acid. He asks, can such formation take place in limited air?

F.—The Experiments of M. de Luca†.

M. de Luca found that, on passing moist ozonous air over potash and potassium, nitrate of potash was formed. He further found that the oxygen given off by plants

* Compt. Rend. xli. † Ibid. 1856.

in sunlight was in many cases ozonous. He aspirated a large quantity of air, in the neighbourhood of vegetation, through carded cotton, and then through sulphuric acid, to wash it. The washed air then passed over potassium, and through a dilute solution of pure potash, when nitrate of potash was formed. When, on the other hand, air in the midst of habitations was operated upon in a similar way, the formation of nitric acid was not observed. M. DE LUCA supposes the air surrounding vegetation, in sunlight, to be ozonous; that by its means the Nitrogen of the air may be converted into nitric acid; and that thus the Nitrogen of the air may be rendered available for assimilation by plants, under the influence of vegetation itself.

G.—The Experiments of M. Harting*.

In 1855, M. HARTING published some criticisms, and the results of some experiments, on the question of the assimilation of Nitrogen by plants. He considered that the Nitrogen of the air might contribute indirectly to vegetation. He attributed a formation of ammonia from the decomposing débris of seeds, &c., and the free Nitrogen of the air, in the case of M. VILLE's experiments; and also supposed that nitric acid might be formed by the oxidation of the atmospheric Nitrogen. The increase of Nitrogen in M. VILLE's plants, and of ammonia in the water of the enclosing apparatus, was taken as proof of such formation of ammonia.

M. HARTING made two sets of experiments, in one of which the plants grew in a limited volume of air, and in the other in a current of air washed free from ammonia—both arranged with a view to avoid the formation of ammonia. He employed enclosing-apparatus somewhat on the plan of M. BOUSSINGAULT and M. VILLE; but he used glass vases, instead of porous pots, for his plants. He grew Beans, Buckwheat, and Oats. After the seeds had germinated, and the plants had protruded a little above the surface of the artificial soil, he covered the latter with a mixture of wax and oil, to shut off the access of air. He further enclosed the stems of the plants in caoutchouc tubes; and inserted other caoutchouc tubes through the waxy coating, for the supply of water. Some of the plants were very vivacious at first; and in the case of the Beans, two began to flower; but then the leaves turned yellow, and the experiment was stopped. His apparatus consisted of tinned-iron pans, varnished, and surmounted by glass shades of 18 litres capacity. There was an aperture for the admission of carbonic acid, another for that of water, and so on.

The result was that the produced plants yielded no more dry matter than was contained in the seeds. M. HARTING considered, therefore, that the determination of the Nitrogen was superfluous. The growth evidently stopped when the supplies of the seeds were exhausted. M. HARTING's general conclusions on the subject were as follow:—

1. Plants absorb salts of ammonia, and nitrates, by their roots.

2. The Nitrogen of the air contributes to the formation of ammonia, and nitrates, in the soil.

3. It is not proved that Nitrogen serves directly for the nutrition of plants.

* Compt. Rend. xli., 1855.

H.—M. A. Petzholdt on the Source of the Nitrogen of Plants*.

In the years 1852 and 1853, M. H. M. Chlebodarow made some experiments on the subject of the assimilation of Nitrogen by plants, at Dorpat, under the direction of M. Petzholdt, who has reported the results of the inquiry.

M. Petzholdt assumes that if plants can appropriate the free Nitrogen of the air, they will not need ammonia; and that if they take Nitrogen from ammonia, the artificial supply of the latter will increase growth.

The experiments were made upon Barley. In 1852, an ignited yellow sand was taken as the soil. To one set of plants, no ammonia was supplied; to a second, carbonate of ammonia was provided in the soil; and to a third, carbonate of ammonia was supplied in the air. Both the crops with an artificial supply of ammonia gave three times as much produce as the crops without such supply. The Nitrogen in the produce was also very much greater, both in percentage, and in actual amount, where the ammonia was used.

In 1853, six sets of experiments were made, and as before, with Barley. The soils consisted of an artificial mixture of clay, sand, and felspar, decomposed by heating with lime. The first set of three pots was provided with this soil alone; the second had, in addition, 0·13 per cent. of bone-ash acted upon by sulphuric acid; and the third had 1·33 per cent., or ten times as much, of the same phosphatic manure. The three other sets were, respectively, so far like the three just described, but in addition ammonia was artificially supplied to the atmosphere in which the plants grew. The phosphatic manure, whether with or without the ammoniacal supply, much increased the produce of both corn and straw. The Nitrogen of the crops was also very much increased in actual amount (though diminished in percentage in the dry substance) by the aid of the phosphatic manure; and the actual amount of Nitrogen was still further increased by the addition of ammonia to the atmosphere of the plants; and the percentage of Nitrogen in the dry substance was also greater where the ammonia was supplied, than in the corresponding cases without it. The experiments without ammonia were made in free air. The Nitrogen in the produce was about seven times that of the seeds where no phosphates were employed; about twelve times that of the seed with the smaller quantity of phosphate; and about twenty times that of the seed with the larger amount of phosphate.

M. Petzholdt considered it difficult to account for the fact of M. Boussingault getting little or no increase of Nitrogen when he grew plants in free air, which must have supplied some ammonia, even though rain and dew were excluded. He thinks the error must be on the side of M. Boussingault.

It is seen that the explanations or conclusions of these several arbitrators are nearly as conflicting as those of M. Boussingault and M. G. Ville themselves.

For ourselves we are free to confess that we are unable to discover, either in the

* Journ. für Prakt. Chem. Band lxv.

differences of plan adopted by M. Boussingault and M. G. Ville, so far as they have themselves described them, or in the results and explanations of other experimenters, any satisfactory solution of the difference of result arrived at. *A priori*, there are reasons for concluding, both from the chemical characters of Nitrogen itself, and from what we at present know of the chemistry of vegetation in other respects, that plants would not assimilate Nitrogen offered to them in the free state. On the other hand—to say nothing of the large total amount of combined Nitrogen actually existing—the statistics of Nitrogen-production show that there is an amount of Nitrogen periodically available for the vegetation of a given area of land, the source of a considerable proportion of which is as yet not satisfactorily explained. The question whether or not the assimilation of free Nitrogen by plants may account for all, or a part, of the otherwise unexplained fixation, is seen to be left in a dilemma almost inexplicable, by the conflicting character of the results that have been recorded relating to it. Yet, as has been already said, upon the decision finally come to in regard to this question, must materially depend the degree of importance to be attached to the investigation of the other actual or possible sources of Nitrogen to plants, which we have briefly noticed. Under these circumstances, it seemed desirable that any opinions we might offer or adopt on this subject should have the support of such evidence as might be afforded by renewed experiment. We proceed, then, to follow up our account of the Nitrogen-statistics of vegetable production, the consideration of the several possible sources of Nitrogen to plants, and the review of the results and opinions of others on some of the points at issue, by a statement of our own experimental evidence in regard to this important question.

PART SECOND.

EXPERIMENTAL RESULTS OBTAINED AT ROTHAMSTED DURING THE YEARS 1857, 1858, AND 1859.

Introductory observations.

In laying this part of the subject before the Fellows of the Royal Society, we shall follow the general order in which the questions involved were presented to ourselves in the investigation. In so doing it will be necessary :—

1. To consider all possible conditions to be fulfilled in order to effect the solution of the main question of the assimilation of free Nitrogen by plants, and to endeavour to eliminate all sources of error in our investigation.

2. To examine a number of collateral questions, which have a bearing upon the points at issue, and to endeavour so far to solve them as to reduce the general solution to that of a single question to be answered by a final set of experiments.

3. To give the results of the final experiments themselves, and to discuss their bearings upon the question which it is proposed to solve by them.

We shall dwell more fully upon the conditions involved in the experiments than upon the numerical results which they have afforded, since the value of these results is so wholly dependent on those conditions, that, if the latter are properly arranged and thoroughly considered, any conclusion with regard to the former will be sufficiently apparent from the numerical results themselves.

In studying the conditions, we shall be obliged to touch upon several collateral points, embracing some questions not necessarily involved in the investigation, and which, therefore, we have not attempted to treat with that fulness which, as distinct questions in vegetable Physiology, they merit. Yet, we think, it will appear that, in the degree in which we have followed them, their discussion is essential to complete the consideration of the main question of the investigation, and that, in relation to it, they possess an interest quite commensurate with the attention we have devoted to them.

These questions are embraced in the following :—

1. The preparation of the soil or matrix for the reception of the plant, and of the nutriment to be supplied to it.

2. The preparation of the nutriment to be supplied to the plant,—embracing that of mineral constituents (as in the ash), of certain solutions, and of water.

3. The conditions of the atmosphere to be supplied to the plant, together with the means of securing them,—involving a consideration of the circumstances affecting the composition of the atmosphere, and of the apparatus used to supply it.

4. The changes undergone by nitrogenous organic matter during its decomposition, affecting the quantity of combined Nitrogen present, in circumstances more or less analogous to those in which the plants were grown in our experiments upon the assimilation of Nitrogen.

5. The action of agents, as ozone, and the influence of other circumstances which may affect the quantity of combined Nitrogen present in connexion with the plant, and yet independent of the direct action of the vital (growing) process.

In considering these five questions, two important series of conditions must be fulfilled :—

1. Those that relate to the growth of the plant,—which must be so arranged as to include all that is necessary for healthy and vigorous growth, *excepting only*, in some instances, such conditions as may depend upon the presence of a supply of combined Nitrogen.

2. Those that relate to our means of measuring the quantity of combined Nitrogen present at different periods of growth,—it being essential that we should be able, with the means of investigation afforded in the present state of science, to ascertain the quantity of combined Nitrogen present with the plant at different periods of its growth, with sufficient exactness to detect any changes that may take place, so as to enable us to refer them to their proper source.

If we succeed in fulfilling all these conditions, we shall have at our command all the data requisite for the solution of the question whether plants assimilate free

Nitrogen. Our preliminary investigations will have enabled us to avoid, or to eliminate, all sources of error due to the incidental circumstances of the research; and the numerical results of a final series of experiments, showing the quantities of combined Nitrogen supplied, and those eventually found in connexion with the plant, will afford the necessary data for the solution desired.

In discussing the conditions involved in the experiments, and the researches undertaken to enable us to estimate the value of those conditions, we shall arrange the subject in such order as will most clearly bring out their bearings upon the main question, rather than according to the order as to time in which they were made. Several collateral experiments were made, to prove that our conditions of growth, provided in soil, atmosphere, and nutriment, were such as we had assumed them to be; for had they not been so, the object of the investigation could not be attained. The time required for the conduct of these collateral experiments, made it necessary that many of them should be performed simultaneously with the investigations the proper conditions of which they were designed to make known.

We shall first consider the arrangement of the main experiments, and the plan and results of the collateral inquiries with a view to show what the conditions of the former should be, and then show how far the conditions assumed for the first year's experiments, and those arranged in the second year, after the results of some of the collateral investigations were known, agree with the conditions indicated by the results of all the collateral investigations taken together.

Section I.—CONDITIONS REQUIRED, AND PLAN ADOPTED, IN EXPERIMENTS ON THE QUESTION OF THE ASSIMILATION OF FREE NITROGEN BY PLANTS.

A.—*Preparation of the Soil, or matrix, for the reception of the plant, and of the nutriment to be supplied to it.*

In considering the subject of the soil to be used, the remarks made above on the necessity of combining the conditions of healthy growth with the simplicity of constitution which would allow of a quantitative estimation of the results obtained, acquire a high degree of importance.

So complicated is the constitution of ordinary soils, and so intimately are the nitrogenous compounds existing within them associated with the other matters, that it is impossible either to estimate the Nitrogen with sufficient accuracy for our present purpose, or to extract it from the soil without entirely destroying the other conditions of vegetable growth. We are, moreover, so entirely ignorant of the character of the organic constituents of soils, of the state in which the principal part of the Nitrogen exists in them, of the changes to which it is subject during vegetable growth and decay, and, more especially, of its relations to vegetable growth, that an ordinary soil could not possibly be used for our purpose.

Our ignorance of the actual constitution of soils, as regards the state of the organic

matter in them, and its relations to the inorganic substances, entirely precludes the possibility of our imitating, by artificial means, a natural soil, so as to include all its conditions excepting a supply of combined Nitrogen.

It is evident, therefore, that if all the conditions embraced in an ordinary soil were essential to vegetable growth, the solution of the question of the assimilation of free Nitrogen by plants would involve difficulties which our means of investigation in the present state of science could not overcome. But the experiments to which attention has been directed in the history of this subject, as well as others, the details of which we shall give further on, show that such is not the case. They show that many of the complicated conditions of an ordinary soil may be entirely dispensed with, so as to bring the examination of it within our means of investigation, and yet to retain all the conditions of healthy growth.

In the experiments of the first year, 1857, two kinds of soil, or matrix, were used.

One was prepared from an ordinary soil, so as more nearly to imitate the usual conditions of vegetable growth. The other was prepared from volcanic pumice, with the view to eliminate certain supposed sources of error which the prepared soil might introduce. It was found, however, in the experiments of 1857, that there was no necessity for this difference of matrix, and hence, in the experiments of 1858, only prepared soil was used.

The soil selected for the preparation of the matrix was a somewhat heavy one (clayey), resting upon chalk, and interspersed with flints. The large stones were removed by picking and sifting; and the clayey lumps were powdered to prevent them from baking into hard nodules during ignition. An attempt to ignite in ordinary clay crucibles was not successful, owing to the reduction of the peroxide of iron to the state of black oxide, and to the formation of sulphides from the reduction of the sulphates present, as indicated by the vapours of sulphurous acid emitted during the ignition, and by the evolution of sulphide of hydrogen on the addition of an acid to the mass after cooling.

The combustion proceeded satisfactorily in a large cast-iron muffle, through which a constant current of air could pass. The ignition was continued until a portion of the soil assumed, on cooling, the red colour due to peroxide of iron, and exhibited no trace of coaly matter. The mass thus prepared was taken from the muffle, and thrown into a large vessel filled with distilled water. The water was rendered highly alkaline by the quantity of caustic lime present. The fluid was decanted, and fresh portions of water added several times during eight or ten days, until all the soluble matter was removed. The residue was then dried, and retained for final ignition before being used. The ferruginous and aluminous character of this soil-matrix pointed to the danger there might be of its acting as a porous body, to promote the formation of nitrogenous compounds independently of vegetable growth, on the one hand, or to absorb and retain the ammonia given to the plant, or that which might be formed from the nitrogenous matter of the seed, on the other.

To ascertain the value of any influence exerted by the soil independently of the plant,

in the manner just indicated, a pot of soil, prepared exactly as for an experiment with a plant, was submitted to the same conditions of air, temperature, moisture, &c., as the pots containing the experimental plants. The result was, that there was no accumulation of combined Nitrogen. The result with the matrix of pumice also showed, compared with that of the soil-matrix, that no error was to be feared from the influence of the latter in absorbing and retaining combined Nitrogen already in connection with the plant.

For our purpose, pure volcanic pumice was used. It was powdered until the mass was quite fine and the largest pieces were about the size of peas. This powder was subjected to long washing in the same manner as the ignited soil. Lastly, it was dried ready for a final ignition before being used.

B.—*The Mineral Constituents added to the prepared Soil.*

In most cases the necessary Mineral Constituents were supplied in the form of the ash of the plant of the description to be grown. In a few instances, where this was not practicable, the ash of some other plant was selected. Weak solutions of sulphates and phosphates, as well as ash, were also sometimes used.

In some instances the ash was obtained by burning a quantity of the entire plant when in seed. In other cases, the seed and the rest of the plant being burnt separately, a mixture of the two ashes was made in such proportion as to represent the composition of the ash of the entire plant.

Thus, in the experiments of 1857, for Wheat a mixture of one part of the grain-ash and six parts of the straw-ash, for the Barley a mixture of one part of the grain-ash and three parts of the straw-ash, and for Beans a mixture of one part of the corn-ash and two parts of the straw-ash was used. In the experiments of 1858, the ash used for these crops was obtained by burning the entire plant. For Clover, the ash of Clover-hay was employed.

In some instances of Leguminous plants the ash was saturated with sulphuric acid, and then ignited, before being used.

Each ash was burnt in a large shallow platinum dish, heated in a current of air, in a cast-iron muffle. The burning was continued until all coaly matter had disappeared. The ash was then preserved, but was always submitted to a final ignition before being used. Examination failed to detect combined Nitrogen in any of the ashes so prepared.

In order that the roots of the plants should find an abundance of mineral matter at the most active period of growth, it was desirable that the matrix should contain as much of such matter as was consistent with healthy development. A consideration of the chemical constitution of soils suggested a proportion of 0·8 to 1·0 per cent. of ash; and this was the quantity added to the matrix for the experiments of 1857; but for those of 1858 only about half as much was employed.

C.—*The Distilled Water.*

The first two-fifths of the distillate from ordinary water was allowed to escape, and the next two-fifths were collected for further treatment. The water so obtained retained traces of ammonia. It was mixed with phosphoric acid, free from nitric acid and ammonia, in such quantity that the amount of acid present exceeded that of the ammonia several thousand times. It was then re-distilled from a copper vessel to which was attached a large Liebig's condenser.

Under these circumstances no ammonia could go over unless it were carried over mechanically, in which case it would be accompanied with several thousand times its own weight of phosphoric acid; and, as no distilled water was used that gave any evidence of the presence of this acid, the amount of ammonia in it, if any, must have been several thousand times less than that to which the term "traces" is applied.

The distilled water was so prepared only a few days prior to being required for use.

All parts of the apparatus, the presence of ammonia in which could possibly affect the result, were, after thorough washing both with ordinary and with common distilled water, finally well rinsed with this pure double-distilled water just before being used.

D.—*The Pots used to receive the Soil, Ash, Plant, &c.*

For the experiments of 1857 common flower-pots were used; their height, and diameter at the top, were each 6 inches, and their diameter at the bottom 3·2 inches; their weight was about 1 lb. Small common white glazed earthenware plates were used as the pans.

Subsequent observation suggested, for the experiments of 1858, the kind of pot, and pan beneath it, represented in Plate XII. figs. 1, 2 & 3.

The Pot, of which fig. 2, Plate XII., represents the elevation, was made of the same material as ordinary flower-pots. It was, however, made as light as possible, and was not baked so hard as the latter generally are. The height, and diameter at the top, were each 5 inches; and the diameter at the bottom was 4 inches. The bottom is perforated with about twenty holes of nearly one-fourth of an inch diameter, as is shown in figs. 1 & 2. There were also two rows of similar holes (A, B, fig. 2) round the sides at a distance of 0·5 to 1 inch from the bottom.

The Pan, represented with the pot placed in it in fig. 3, Plate XII., is made of hard-baked and well-glazed stone-ware. It is 1·5 inch deep and 5·2 inches in diameter at the bottom. At the top it is curved inwards (A, B, fig. 3), so as to adapt its upper rim to the sides of the pot.

These arrangements of pot and pan afford several advantages, for the purposes of the investigation, over those adopted in 1857. The surface for evaporation is less in proportion to the volume of soil. The facilities for the exit of roots, and for the access of air, are, on the other hand, greater. The pan affords room for an abundance of water,

in which the roots develop luxuriantly*. Yet this water does not evaporate so freely as it otherwise would do, owing to the inward curve of the top of the pan, which also serves to protect the roots distributed through the water from the direct action of sunlight. All the conditions of growth are thus attained with a minimum of evaporation from all sources excepting through the plant itself; and a drier atmosphere is maintained. Consequently evaporation through the plant is favoured, and hence the conditions are provided for a constant supply to the plant of all the mineral and gaseous substances in solution in the fluid of the soil and pan.

E.—*Final preparation of the Soil, Ash, and Pot, for the Plant.*

The soil and ash, each prepared as described in the foregoing subsections, and the pot, also as described above, were simultaneously heated to redness; and the soil and ash, whilst red-hot, were mixed together in the red-hot pot, which was placed upon a red-hot brick over sulphuric acid. The pot and contents were then covered with a large glass shade, and left to cool.

The soil, as in its former preparation, was heated in a cast-iron muffle, from which it was removed with a small iron shovel adapted to the purpose, and heated to redness before being used.

Four or five pots were heated together, one inside the other, the top and bottom ones of which almost invariably broke, either on the application of the heat, or on removal from the fire; so that only about half of those operated upon were finally available for use.

From $2\frac{1}{2}$ lbs. to 3 lbs. of ignited soil were put into each pot; but in the experiments of the second year, 1858, the lower part of the pot was first filled, to the depth of about 1 inch, with very coarsely broken-up red-hot flint. In 1857, about 14 grammes of ash, and in 1858 about 7 grammes only, were used for each pot. The greater portion of the ash was mixed with the lower layers of the soil; but some was distributed through the whole of it.

After cooling down sufficiently, the shade was removed, and about 500 cub. centim. of distilled water, prepared as described in subsection C, were added to the soil of each pot, this being as much as it would absorb. Then, after a lapse of ten to twenty hours, the seeds or plants were put in.

F.—*The Seeds and Plants taken for experiment.*

In all the experiments recorded, the plants were grown directly from *seed* sown in the soils prepared as above described.

In every case, seed of the best quality was taken, which was kindly presented to us for our purpose by the Messrs. Thomas Gibbs and Co., of Half-Moon Street, Piccadilly,

* See Table, and general remarks at p. 524; also notes of root-development of Wheat No. 6 (1857), p. 553, Wheat No. 1 (1858), p. 560, and Wheat No. 9 (1858), p. 569.

Seedsmen to the Royal Agricultural Society of England, who bestowed much labour and attention upon the selection. From the quantity of each kind received, the largest and the smallest were picked out, as were also any that did not look quite healthy. Given numbers of the remainder were then weighed, and the average weight, per seed, was calculated. A few seeds, each weighing as nearly as possible the mean weight, were then selected for planting.

In order to estimate the quantity of Nitrogen in the seeds sown, in some cases a quantity of seeds equal in weight and number to those sown was submitted to analysis. But the difficulties of grinding, without loss, so small a quantity, and the consideration that one small quantity might differ more in composition from another such quantity, than either would from the average composition of a large number of well-selected seeds, led us generally to estimate the Nitrogen in the seeds sown from the percentage of it found in the mixture of a large number ground up together.

The seeds selected for growing were sown in the pots of soil prepared as already described, to the depth of about 1 inch below the surface. With large seeds such as Beans, it was necessary that care should be taken so to deposit them that the radicle and plumule should each take its natural direction. If this precaution was neglected, the seed was liable to be raised out of the soil after sprouting, which involved the inconvenience of opening the apparatus in which the plant was enclosed, in order to re-bury the seed.

In some cases, as soon as the seeds were sown the pots were removed from over the sulphuric acid, and placed at once beneath the large glass shades which were to serve as the enclosing apparatus. In other instances, the pots were first placed under other shades, luted by mercury or sulphuric acid, and standing in the laboratory, and then, after a few days, they were removed to their final position.

G.—*The Atmosphere supplied to the Plants.*

As regards the essential conditions of growth, and the circumstances associated with it, which must be kept within the control of our means of investigation, the same remarks apply to the atmosphere, though with less force, as have already been made in reference to the soil (subsection A, p. 470).

It is true that the constitution of the atmosphere is less complicated, and that we are much better acquainted with it than we are with that of ordinary soils ; yet the extreme mobility of the atmosphere renders the presence in it of exceedingly small quantities of substances calculated to influence vegetable growth much more dangerous in quantitative experiments on vegetation than would be their presence in the soil. Thus the presence of gaseous impurities, and of solids mechanically suspended, in the atmosphere cannot be overlooked. And hence, although it is not necessary to submit the natural atmosphere to such radical changes as those to which the natural soil must be subjected, some measures must be taken to exclude the sources of error to which allusion has just been made.

As in the case of the soil, so in that of the atmosphere, the only essential conditions to be attained are such as are required for healthy growth, and as will at the same time enable us to estimate the amount and the sources of the combined Nitrogen coming within the reach of the plant by its means.

In consequence of the mobility of the atmosphere above referred to, it was necessary to exclude the experimental plants from its free access. The quantity of ammonia in the air is, however, so very small, that, provided the atmosphere of the enclosing apparatus were allowed to remain unchanged throughout the period of an experiment, the amount of combined Nitrogen so coming within the reach of the plant might be altogether neglected. Nor, so far as regards the nitrogen and oxygen of the air, is there any necessity for change; but, owing to the peculiar circumstances of temperature and of moisture to which the air of the apparatus is subjected, conditions more closely allied to those of ordinary vegetation are attained by a frequent change of atmosphere. The large quantity of air which thus becomes involved in an experiment precluded the idea of neglecting the consideration of the combined Nitrogen which it contains. It devolved upon us, therefore, either to determine the total amount of combined Nitrogen in the air before and after it came in contact with the plant, or to free the air from combined Nitrogen before admission into the enclosing apparatus. The latter alternative was adopted as the most simple; and the manner in which the object was effected will appear from the following description of the apparatus employed.

H.—*The Apparatus used to enclose the Plants, and to supply them with Air, Water, Carbonic Acid, &c.*

Plate XIII. represents the entire apparatus as used for each separate experiment in 1857; and fig. 1, Plate XIV., that used, also for each separate experiment, in 1858, in which, as will be seen, several important modifications of the arrangement adopted in 1857 were made.

The same letters of reference apply to the two so far as the parts are alike; and where there has been any modification in the arrangement in 1858, as compared with that in 1857, the same letters represent the parts of the apparatus used for the same purpose in each, with the exception, that those which apply to the modification of the apparatus in 1858, are distinguished by a dash, thus ′.

A, Plate XIII. (and fig. 1, Plate XIV.), represents a large stone-ware Woulfe's bottle, 18 inches in diameter and 24 inches high.

B, C, and E are glass Woulfe's bottles of 30 ounces capacity.

F is a large glass shade, the dimensions of which were, in most of the experiments, diameter 9 inches, and height 40 inches; in other cases the dimensions were, diameter 16 inches, and height 28 inches.

a represents the cross section of a leaden pipe $1\frac{1}{2}$ inch in diameter, which is in connexion with a reservoir of water, not shown. This pipe passes over all the vessels A (of

which there is one included in the apparatus for each separate experiment) in a direction at right angles to the plan of the figure. It is connected with each vessel A by means of a tube *a b*, in which is fixed a stopcock, to open and shut the connexion between the water-supply tube *a* and the vessel A.

c d e, Plate XIII., is a leaden exit-tube for air. *c′ d′ e′*, fig. 1, Plate XIV., is the corresponding tube in the apparatus of 1858, which is enlarged at the point *c′* and downwards until it opens into the vessel A, thus allowing another, *q′ r′ s′*, to pass through it and down to the bottom of the vessel A, as indicated by the dotted line. This tube *q′ r′ s′* is a half-inch safety-tube, opening externally at *q′*, and in the apparatus of 1858 replaces the tube *q r s* shown in Plate XIII.

The bottles B and C are filled to the depth of $2\frac{1}{2}$ inches with sulphuric acid of sp. gr. 1·85.

The tube D D is about 3 feet long and about 1 inch in diameter, and is filled with fragments of pumice saturated with sulphuric acid. At *f*, *f*, in this tube, are small indentations to prevent the sulphuric acid from draining against the corks.

The Woulfe's bottle E contains a saturated solution of ignited carbonate of soda.

g h is a bent and caoutchouc-jointed glass tube, connecting the interior of the Woulfe's bottle E with that of the large glass shade F.

i k, better indicated in fig. 2, Plate XIV., is the exit-tube for the air, connecting the interior of the shade F with an eight-bulbed apparatus M, containing sulphuric acid.

w w, Plate XIII., is a block of slate 12 inches square and $3\frac{1}{4}$ inches thick, in which is a circular groove, half an inch wide and 2 inches deep, adapted to the diameter (9 inches) of the glass shade F, the bottom of which rests in it. The groove is filled with quicksilver, which shuts off the communication of the external air with the interior of the shade. It is widened and deepened at four equidistant points, to admit of glass tubes passing underneath the shade. Two of these tubes, *g h* and *n o*, are shown in Plate XIII., and *g h* also in fig. 1, Plate XIV., *n o* being there replaced by *n′ o′*. The other two are at right angles to these, and are best seen in the vertical section of the shade and lute, fig. 2, Plate XIV., lettered *u v* and *i k* respectively. The tube *u v* is for the supply of water to the plant; and the tube *i k* is for the exit of the air, from which it passes outwards through the sulphuric acid in the eight-bulb apparatus M. This vertical section of the shade and lute is at right angles to the view of them in fig. 1, Plate XIV.; and from it a judgment may be formed of that of the shade and lute of Plate XIII., as well as of the corresponding tubes to those last described, in the apparatus of 1857.

The tube *n o*, Plate XIII., passing from the outside, beneath the shade, and extending to the surface of the mercury in the groove within the shade, is for the purpose of withdrawing condensed water. In the apparatus for 1858, the arrangement for this object is rather different. Thus O, fig. 1 (and fig. 2), Plate XIV., is a bottle into which passes a tube *n′ o′*, opening into the bottom of the lute *w′ w′* by means of a hole at *n′*,

seen better in figs. 4 & 5, Plate XII., which represent, respectively, the plan and the vertical section of the glazed stone-ware lute-vessel used in 1858. Another glass tube (t') passes to the bottom of the vessel O (figs. 1 & 2, Plate XIV.), for the purpose of withdrawing the condensed water which collects in it.

The plan of the stone-ware lute-vessel used in 1858 (fig. 4, Plate XII.) shows the groove for the mercury, the four widened and deepened points of it for the passage of the tubes under the shade (of which however three only were used), and the hole n' at the bottom, for the reception of the tube for carrying off the condensed water. This lute-vessel is made of hard-baked and well-glazed stone-ware, and is, in fact, simply a shallow dish with double concentric sides, the space between which latter forms the groove for the reception of the shade and of the mercury luting, and for the passage of the tubes. Figure 5, Plate XII., is a vertical section of the stone-ware lute-vessel, from A to B, fig. 4, through two of the widened and deepened portions of the groove, and through the hole n'. Figure 6, Plate XII., is also a vertical section of the lute-vessel, but from C to D, fig. 4.

The Woulfe's bottle T, fig. 1, Plate XIII., and T′, fig. 1, Plate XIV., is for the supply of carbonic acid, and will be referred to, more fully, in the following subsection I.

I.—*Use of the Apparatus.*

If the stopcock below a (fig. 1, Plate XIII., and fig. 1, Plate XIV.) is opened, water flows into the vessel A from a large reservoir with which the leaden tube a is in connexion. As the pressure increases, the water rises in the safety tube $q\ r\ s$, or $q'\ r'\ s'$ above the level in the vessel A, and at the same time the air begins to escape by the tube $c\ d\ e$, or $c'\ d'\ e'$, to force its way through the sulphuric acid in the bottles B, C, then to traverse the tube D D, containing the pumice saturated with sulphuric acid, to bubble through the solution of carbonate of soda in E, and finally to enter the shade F by the bent and jointed tubes g, h; and from the shade it passes out through the tube $i\ k$ and the bulb-apparatus M containing sulphuric acid, into the external air.

The minimum pressure required to produce this passage of air, expressed in the height of a column of mercury which it would sustain, is equal to the sum of the products obtained by multiplying the height of each fluid through which the air has to pass by the sp. gr. of the same, divided by the sp. gr. of mercury, or

$$[(2{\cdot}5+2{\cdot}5+1{\cdot}0)\times 1{\cdot}85+2{\cdot}5\times 1{\cdot}2]\tfrac{1}{13{\cdot}6}=1{\cdot}037 \text{ inch},$$

in which 1·2 is the sp. gr. of the carbonate-of-soda solution.

The difference between the height of the water in the vessel A and in the safety-tube $q\ r\ s$, or $q'\ r'\ s'$, must always be equal to the weight of the mercury column obtained in the manner just indicated, multiplied by the sp. gr. of mercury.

If the difference between the height of the highest points of the tubes $q\ r\ s$ and $c\ d\ e$, fig. 1, Plate XIII. (that of the former being the higher), be less than the minimum height

just referred to, then the water must flow out of A through the safety tube q. r, s before it can pass through the tube c, d, e, into the bottle B. In accordance with this consideration, the safety-tubes for the apparatus of 1857 were arranged as shown in Plate XIII. (q, r, s) ; but, owing to occasional leakage of the joints at the top of the vessel A, this principle could not be relied upon ; and hence the arrangement shown in fig. 1, Plate XIV., was adopted in the experiments of 1858.

The height from the top of the vessel A to r' (fig. 1, Plate XIV.) was 12 inches, which is sufficient to allow the whole of the air to pass out of the vessel A, whilst the great height of d', of the tube c', d', e', entirely prevented the water from passing over into the bottle B,—an accident which unfortunately happened on a few occasions with the apparatus of 1857.

When the vessel A was full of water, it was drawn off by a cork-hole at the bottom, air being at the same time admitted by the tube x at the top of the vessel.

The minimum pressure upon the glass shade F would be

$$\frac{1{\cdot}0 \times 1{\cdot}85}{13{\cdot}6} = 0{\cdot}136 \text{ inch},$$

in which 1·0 is the difference between the height of the lowest and the highest level of the sulphuric acid in the bulb-apparatus M. Experiments showed, however, that owing to friction, &c., the maximum pressure on the inside of the glass shade would be raised to double the above estimated minimum.

The plants were supplied with water, as already said, through the tube $u\,v$, shown best in fig. 2, Plate XIV. At first fresh distilled water was supplied; but as soon as a sufficient quantity of condensed water had run through the tube $n'\,o'$ and collected in the bottle O, this was drawn off by means of the tube t' to water the plant when required. In the experiments of 1857, the condensed water was drawn off from time to time, from the surface of the slate and mercury, by means of the tube $n\,o$.

All the Woulfe's bottles were made as air-tight as possible by means of very good corks. Those of the bottles E, Plate XIII., and fig. 1, Plate XIV., and also those of O, fig. 1, Plate XIV., were, however, covered with a cement, composed of eight parts gutta percha, twelve parts common rosin, and one part Venice turpentine, well melted together. The glass tube $n'\,o'$ was also fixed into the lute-vessel $w\,w$, at n', with this cement.

In the experiments of 1857, tubes of unvulcanized caoutchouc, made by ourselves from the sheet, were used for the various joints indicated in the figures ; but as these soon became unsound under the influence of the atmospheric changes to which they were exposed, tubes of vulcanized caoutchouc were substituted in 1858. The ends of the glass tubes o and u, Plate XIII., and of the tubes t' and u, fig. 1, Plate XIV., were fitted with pieces of caoutchouc tubing into which pieces of solid glass rod were fixed as stoppers.

In 1857, twelve such sets of apparatus, and in 1858 a larger number, were employed. The whole were arranged side by side, on stands of brickwork erected for the purpose,

in the open air, and were protected from rain, or the too powerful rays of the sun, by a canvas awning which could be drawn down over them, or withdrawn, at pleasure.

In 1858, in addition to the sets of apparatus above described, two glazed cages, such as were used by M. G. VILLE in his experiments, and which he kindly sent over to us for the purpose, were employed.

J.—*The supply of Carbonic Acid to the Plants.*

Owing to the small proportion of carbonic acid in the atmosphere, and to the fact that a part of it would be absorbed in passing through the apparatus just described, it was necessary to give a supply of it to the plants artificially. It was obtained by the action of chlorhydric acid upon fragments of marble in the vessel T, Plate XIII., or T′, fig. 1, Plate XIV.

In regulating the supply of carbonic acid, the points to be observed were, to keep the proportion in the enclosed atmosphere below that in which it would prove injurious to the plants, and at the same time to provide a sufficient quantity for the demands of vegetation at the most active periods of growth.

BOUSSINGAULT found * that the air surrounding a plant might, consistently with healthy growth, contain 8 per cent. of carbonic acid. This amount, then, on the one hand, and the very small quantity in the atmosphere which is sufficient for natural vegetation (about 0·04 per cent.), on the other hand, afford us limits between which a wide range is allowed for variation.

Calculation showed that a minimum quantity of 0·2 per cent. of carbonic acid in the air of the enclosing apparatus would supply 5 cubic inches of the gas within the shade at one time, corresponding to 0·0439 gramme of carbon—a quantity which, maintained daily throughout the sunlight, would be very much more than was required by the plants.

It is obvious, therefore, that a variation in the amount of carbonic acid in the atmosphere of the plants between 4·0 per cent. and 0·2 per cent. would be very safely within the limit suggested by the experiment of BOUSSINGAULT as the maximum, on the one hand, and that indicated by the above considerations as the minimum desirable in the experiment, on the other.

A question arises as to the influence which the *changes* in the proportion of carbonic acid in the air, between the assumed limits, may have upon the plant. In reference to this point, it may be mentioned that our own experiments upon the nature of the gas in plants (some of the results of which will be given further on) appear to show that the changes in the proportion of the carbonic acid in the air of the cells and intercellular passages, and in that in the fluids of the stem, are much greater, and more rapid than those which can take place in the atmosphere of our apparatus. In addition to this, may be stated the fact that plants derive much of their carbonic acid from aqueous solution absorbed by the roots; and most probably the remainder is absorbed by the fluids of the plant before influencing its growth. These absorptions can take place but

* Mémoires de Chimie Agricole et de Physiologie, 1854, p. 441.

slowly ; so that somewhat rapid variations in the proportion of carbonic acid in the atmosphere surrounding the plant, will be accompanied by much less variation in the proportion of carbonic acid within the plant. The latter will, therefore, be a slightly varying mean between amounts corresponding to the foregoing extremes.

From the above considerations, it appeared probable that there would be no danger in so supplying carbonic acid to the atmosphere of the plants as that its proportion should reach its maximum in a short time, and then, by the passage of air, gradually fall again to the minimum. A few trials, adding different quantities of chlorhydric acid to the vessel T, Plate XIII. (or T′, fig. 1, Plate XIV.), containing marble, enabled us to ascertain the proper quantity to add, to provide about 4 per cent. of carbonic acid in the shade F when air was not passing. Then passing air, it was found that the proportion of the gas was never reduced below that which we have above assumed as the proper minimum. In practice a little more chlorhydric acid than the amount so determined was used ; and then the passage of the air was commenced simultaneously with the addition of the acid. Repeated analyses of the air in the enclosing apparatus showed that, operating in this way, our assumed limits for the maximum and minimum proportions, respectively, of carbonic acid were not passed.

The volume of the air passed through the apparatus daily, was that of the vessel A, Plate XIII., and fig. 1, Plate XIV., and was equal to about 2·5 times that of the enclosing shade F.

K.—*Advantages of the Apparatus above described.*

The advantages, for the purpose in question, of the plan of apparatus which has been described, over those of several of the forms that have been suggested or used by others, may be very briefly stated.

1. When once ready to receive the plant, the use of the apparatus is extremely simple and easy. It is only necessary to place the pot containing the soil, seed, &c., with its pan, in the stone-ware lute-vessel, to pour mercury into the groove, to arrange the several tubes, and to put on the shade. The plant is then entirely excluded from all external sources of combined Nitrogen ; and, in case of its being necessary to open the vessel for any purpose, this can be done with great facility.

2. By means of the arrangement of the bottle O (fig. 1, Plate XIV.), the water which condenses within the shade is removed from the atmosphere of the plant as soon as it collects. The small pan in which the pot stands (fig. 3, Plate XII.), with its inward-turned sides, allows of a store of water being kept beneath the plant which is at the same time protected from free evaporation. The vessel O holds as much water as can be evaporated from the plant and soil during several days. The supply of water to the plant is exceedingly easy and simple, it being only necessary to remove that which has collected in the bottle O by means of the tube *t′*, and to pour it in at *u* (figs. 1 and 2, Plate XIV.). [In the arrangement for the experiments of 1857 the condensed water collected on the surface of the slate, until removed by means of the tube *n o*.]

3. A simple glass shade is liable to introduce fewer sources of error than a complicated metallic framework with panes of glass cemented into it. The shade is easier to clean before commencing the experiment, and it is less likely to retain, at the termination of it, any of the combined Nitrogen, either derived from the plant, or from that which has been supplied during growth. Lastly, the presence of oxidizable metallic surfaces, affording a possible quantity of nascent hydrogen which might form ammonia with the Nitrogen of the air, is avoided.

4. There is no organic matter present which can affect the result of the experiment. The only organic matter within the shade is that of a thin coating of the gutta-percha cement which has been described, by which the tube n' (fig. 1, Plate XIV.) is fixed into the hole n' (fig. 4, Plate XII.) at the bottom of the stone-ware lute. On analysis this cement was found to contain from 0·10 to 0·15 per cent. of Nitrogen. Hence, if the whole quantity of the cement in contact with the condensed water became decomposed, and yielded up its Nitrogen in such a manner as to become a product of the experiment, it would only so yield a few tenths of a milligramme of Nitrogen; but experiment proved that it did not suffer sensible decomposition when subjected, during a whole year, to exposure in the open air.

5. In the passage of the air through the apparatus, the excess of pressure was upon the inside, instead of, as in the experiments of others, upon the outside of the enclosing vessel. In experiments of the kind in question in which the apparatus is exposed to the open air, and so subjected to climatic vicissitudes during a considerable period of time, the ordinary means of securing tightness in the laboratory cannot be depended upon; and an apparatus proved to be tight at one time may, as the result of a variety of causes beyond our control, be subject to leakage at another. But a leakage from the inside of the apparatus outwards cannot affect the result of our experiment; whilst a leakage in the opposite direction might introduce combined Nitrogen from the external atmosphere. In the arrangement which has been described, the excess of pressure is always on the inside during the passage of the air; and when the air is not passing there cannot be any important amount in the opposite direction due to changes of temperature and barometric condition, for it can never exceed that required to drive the air inwards through the bulb-apparatus M (Plate XIII., and fig. 1, Plate XIV.), which is altogether insignificant.

6. That part of the apparatus which would be the most liable to leak, and which would be the most damaged by pressure, is subjected to the minimum amount of it. The entire pressure required to force the air through the apparatus, independently of that necessary to overcome friction, is

$$5 \times 1{\cdot}85 = 9{\cdot}25 \text{ inches of water}$$

to pass through the sulphuric acid in the bottles B and C (Plate XIII., and fig. 1, Plate XIV.), and

$$2{\cdot}5 \times 1{\cdot}2 = 3{\cdot}0 \text{ inches of water}$$

to pass through the solution of carbonate of soda in the bottle E, and

$$1 \times 1{\cdot}85 = 1{\cdot}85 \text{ inch of water}$$

to pass through the sulphuric acid in the bulb-apparatus M, equal a total of 14·10 inches water, or a minimum pressure of about 0·5 lb. per square inch. Direct experiment with a manometer showed, however, that the entire pressure, minus that due to the sulphuric acid in the bulb-apparatus M, might, owing to friction, &c., amount to 0·8 lb. per square inch. This would give a lateral pressure upon the sides of the glass shade of about 900 lbs., if the current of air were produced by aspiration instead of forcing—a condition which would be incompatible with the safety of the vessel. In the mode of experimenting adopted, however, the only pressure exerted upon the glass shade was the amount requisite to force the air through the bulb-apparatus M.

It remains to consider the influence upon the air of its contact (in the vessel A) with the water employed to force it through the apparatus. This can be of three kinds :—

1. The proportions of nitrogen and oxygen may be slightly affected by absorption, under the influence of the slightly increased pressure to which the air is subjected.

2. The air may lose its carbonic acid.

3. It may become more or less saturated with aqueous vapour.

The increase of pressure to which the air is subjected in the vessel A is so slight, and the time in which it is there in contact with the water is so short, that the total amount of oxygen and nitrogen absorbed by the water must be very small ; and, since any change in the constitution of the total amount of air will be dependent on the ratio of the absorption coefficients of oxygen and nitrogen on the one hand, and on the ratio of the quantities of these gases in the air on the other, it will be very much less than in the actual amount of air absorbed ; it will in fact be too small to be of any importance.

The whole of the carbonic acid of the air may be absorbed by the water ; but as arrangements are made for the artificial supply of it, this is of no consequence.

The amount of water taken up by the air in the vessel A would at first sight appear to be of more importance. But the time during which the air is in contact with the water in the vessel A is very short, and probably too short for its saturation ; it must lose most or all of its acquired water in passing through the sulphuric acid in the bottles B and C, and over the pumice saturated with sulphuric acid in the tube DD, whilst the redried air passes too rapidly through the carbonate of soda solution in the bottle E for re-saturation ; and lastly, as the air in its previous course through the apparatus will be cooler than within the shade, it will not be so near its point of saturation in the latter as it may be before it reaches it.

L.—*Adaptation for healthy growth of the conditions of experiment adopted.*

We have thus far discussed the possible sources of error in an experiment on the question of the assimilation of Nitrogen by plants, so far as regards the soil, the inorganic nutriment, and the air, to be provided for the plant, and we have pointed out the means

adopted to avoid them. From known considerations with regard to the requirements in the soil and inorganic nutriment on the one hand, and in the atmosphere of the plants on the other, and in all combined, we have concluded what are the proper conditions of vegetable growth. It remains, however, to appeal to the results of direct experiment, to show that our adopted conditions possess the value which we have assumed them to have.

A pot of good garden soil, capable of supporting luxuriant vegetation in the open air, was sown with Wheat, Barley, and Beans, and then placed under one of the experimental shades, and submitted to exactly the same atmospheric conditions as those provided in the experiments on the assimilation of Nitrogen. The result was, exceedingly luxuriant growth (see Records of growth in Appendix, Experiment No. 12, of "Plants grown in 1857," fig. 13, Plate XV.; and also Experiment No. 15, of "Plants grown in 1858"). It was thus proved that the *aërial* conditions supplied in our experiments were adapted for healthy growth.

When pots of soil, prepared precisely as has been described above, were sown with seed and combined Nitrogen artificially supplied, vigorous growth was the result. Hence it was shown that the conditions of *soil* were properly selected.

Section II.—OTHER CONDITIONS OF EXPERIMENT, REQUIRING COLLATERAL INVESTIGATION.

There remain to be considered several conditions which might affect the result of a quantitative experiment on the assimilation of Nitrogen by plants, dependent upon the reciprocal action of the air and the soil, with or without the connexion of the plant.

The following conditions possibly affecting the result of such an experiment, due to the mutual action of the soil, air, and organic matter of the plant, require to be considered :—

1. The influence of ozone, either within the cells of the plant, or in connexion with it, in promoting the formation of nitrogenous compounds from free Nitrogen. The influence of ozone in promoting such formation within the soil, either directly, or in connexion with the organic matter of the plant.

2. The decomposition of nitrogenous organic matter, in relation to the question whether there be an evolution of free Nitrogen in the process.

3. The formation of nitrogenous compounds, through the mutual action of nascent hydrogen evolved by decomposing organic matter, and free Nitrogen.

A.—*General considerations in regard to the possible influence of Ozone on the supply of combined Nitrogen to growing plants.*

The consideration of Ozone in connexion with the plant suggests the possibility of its presence in two distinct ways. It may occur within the cells and intercellular passages of the plant, either in the gaseous state or in solution, or it may be simply around the plant, without existing within its structures.

With regard to the origin of Ozone in connexion with the plant, it may be a product

of the action of the sun's rays, by virtue of which carbonic acid is decomposed, and oxygen evolved. Or, it may result from other causes, to which we shall refer presently.

In order to ascertain how far the presence of Ozone within the plant may have a bearing upon the point at issue, we have attempted to solve, by experiment, the following questions:—

1. Is there, during the growth of plants, Ozone within the cells or intercellular passages?

2. If Ozone be present within the structures of the plant, is it in circumstances in which it would be likely to oxidize free Nitrogen into any of its oxygen compounds?

3. Is Nitric acid present in the living cells of any plant of which it is not a natural product of growth?

In a number of experiments which we have made upon the gases obtained by exhausting plants placed in water freed from air by boiling, no Ozone was perceptible. Another series of experiments upon the oxygen evolved from plants immersed in water saturated with carbonic acid gave similar results.

In the latter series about 1 ounce of the green plant was placed in 500 cub. cents. of carbonated water, and the whole subjected to sunlight. The decomposition of carbonic acid commenced almost immediately, and the evolution of gas was rapid. In this way 100–200 cub. centims. of gas were obtained, which contained sufficient oxygen to inflame a glowing taper; yet no trace of Ozone was manifested on placing test-paper in the gas. That evolved from Wheat, Barley, Oats, Beans, and Clover behaved alike in this respect. Granting that these experiments may not be conclusive for all conditions of the decomposition of carbonic acid by plants, that under certain circumstances Ozone may exist within the vegetable cells and the passages between them, and that it is possible that some of the oxygen of the decomposed carbonic acid may at times appear as Ozone, still, it is difficult to see how it can exert any oxidizing influence upon the free Nitrogen within the plant, under the peculiar circumstances in which it must come in contact with it.

In order to study more fully the circumstances, and to examine, in some detail, the value of the oxidizing and reducing forces operating in the vegetable organism, in the different conditions to which it is subjected during growth, a number of experiments have been made upon plants, under a variety of conditions more or less analogous to those of ordinary growth. As the results of these investigations are too extended in their bearings for full consideration in the present Paper, and are, moreover, not yet sufficiently complete for publication, we shall give here only such of them as bear upon the point now in question.

It is obvious that the formation of Nitric acid, by the mutual action of Ozone and free Nitrogen within the plant, will be dependent upon the activity of the oxidizing power of the Ozone, and on the intensity of the reducing power of other substances in contact with the Nitrogen to be oxidized.

The investigations of Schönbein and others appear to show that, under certain circumstances, nitric acid may be formed by the mutual action of Ozone and free Nitrogen. The question for our consideration here is, whether these circumstances are presented in the cells of plants, and in the passages between them, during growth? The subject of the relation of Ozone to organic matter is obviously too extensive for anything more than a passing consideration here; but we may refer to the well-known intense action of this peculiar body upon organic matter generally, by which carbonic acid is formed, and the Ozone destroyed. It is well known that Ozone is rapidly destroyed if kept in contact with phosphorus or any other reducing substance. If such conditions for the destruction of Ozone exist within the plant, the probability that it can there oxidate free Nitrogen, and so form nitrates, would appear to be exceedingly small. The actual conditions within the plant in regard to the points in question may be most efficiently studied by the examination of the gases they contain, under various circumstances. We proceed, therefore, to notice some of the results of such an examination.

B.—*Composition of the Gas in Plants.*

Experiments, Series 1.

Plants, or parts of plants, were put into a flask filled with water that had previously been well boiled to remove all air from it. A cork, through which a bent glass tube was passed, was then pressed into the flask, so that the tube was filled with the displaced water. The flask was then placed over a lamp, the water boiled, and the water and gas driven over collected over mercury, the boiling being continued until the water distilled over raised that first driven out with the gas to the boiling-point. The vapour thus produced expelled most of the water collected over the mercury. In this way the gas driven out from the plant at the boiling-point was obtained. The following Table (I.) shows the composition of the gas collected under these circumstances. It is seen that Nitrogen and Carbonic acid only were present.

Table I.—Showing the Percentage Composition of the Gas evolved from plants, in water, on continued boiling.

Date (1857).	Description of plant operated upon.			Per cent.		
	Plant.	Part of plant.	How manured, &c.	Nitrogen.	Oxygen.	Carbonic acid.
May 6.	Wheat.	Whole plant.	Mineral manure	45·47	0·0	54·53
May 2.	Wheat.	Whole plant.	Mineral manure	46·29	0·0	53·71
May 2.	Wheat.	Lower part.	Mineral manure	57·00	0·0	43·00
May 2.	Wheat.	Whole plant.	Mineral and Ammoniacal manure	39·14	0·0	60·86
May 2.	Wheat.	Upper part.	Mineral and Ammoniacal manure	37·53	0·0	62·47
May 6.	Bean.	Whole plant.	Mineral manure	62·79	0·0	37·21
May 6.	Bean.	Whole plant.	Mineral manure	63·80	0·0	36·20

Other experiments gave similar results, all tending to show that the reducing power

of the vegetable cells, dependent on the character and conditions of the carbon compounds they contain, was sufficient, under the circumstances specified, to consume all the oxygen (or ozone) that might be present. But the high temperature at which the experiment was conducted must have tended very much to increase this action. In subsequent experiments a different plan of operation was adopted, not open to the same objection.

Experiments, Series 2.

In these experiments, as in all those subsequently referred to, the plants were put into a tall glass vessel (fig. 7, Plate XII.) 1·75 inch in diameter, and 14 inches in height. The mouth of this vessel is fitted with a long cork, previously well boiled in bees'-wax. Through the cork, two glass tubes, *a* and *b*, are inserted. The vessel being filled with water well boiled and then cooled without access of air, the plant is put in and well shaken to remove adherent air-bubbles. The cork, with its two tubes, is then forced in, taking care that both the tubes become filled with water and that no air remains in the vessel. As a further security for tightness, a piece of wide and thick caoutchouc tubing may be drawn over the neck of the vessel, projecting upwards a little above the cork, and then the cup thus formed partly filled with melted wax, forming a layer over the cork and its joints. A funnel is then attached to the tube *b*, by means of a caoutchouc tube which can be closed by a strong pinch-cock. Water being admitted through the funnel into the tube *b*, the tube *a* becomes filled, and it is then brought into connexion, by means of a glass tube and caoutchouc joint fitted with a pinch-cock, with a vessel filled with quicksilver. The connexion being opened, the quicksilver is allowed to flow from the vessel by means of a long tube of more than barometric length fitted into the lower part of it, thus forming a Torricellian vacuum in the mercury vessel. The gas from the plant passes over into this vacuum, and by a simple arrangement is collected in a eudiometer tube for examination.

The following Table shows the amount and composition of the gas obtained from different plants, in the shade, in the manner above described.

TABLE II.—Showing the amount and the composition of the Gas given off by plants, in the shade, into a Torricellian vacuum.

Date.	Description.		Total Gas collected, cub. cents.	Per cent.			
	Part of Plant.	How manured, &c.		Nitrogen.	Oxygen.	Carbonic acid.	Oxygen and Carbonic acid.
			Wheat; 1858.				
June 16.	Whole plant	Mineral manure	57·0	77·72	2·28	20·00	22·28
June 17.	Whole plant	Mineral manure	55·3	77·94	5·06	17·00	22·06
June 16.	Whole plant	Mineral and Ammoniacal manure	57·0	78·60	1·75	19·65	21·40
June 16.	Whole plant	Mineral and Ammoniacal manure	55·7	77·38	3·23	19·39	22·62
June 16.	Whole plant	Mineral and Ammoniacal manure	65·7	82·50	0·30	17·20	17·50
			Barley; 1857.				
June 24.	Whole plant	Unmanured	8·6	85·12	3·93	10·95	14·88
June 24.	Whole plant	Unmanured	20·9	81·48	1·97	16·55	18·52
			Beans; 1858.				
June 17.	Whole plants coming into Flower	Unmanured	54·3	79·74	5·16	15·10	20·26
June 17.	Whole plants coming into Flower	Unmanured	41·5	86·74	4·10	9·16	13·26
June 17.	Whole plants coming into Flower	Ammoniacal manure	52·5	80·38	4·38	15·24	19·62
June 17.	Whole plants coming into Flower	Ammoniacal manure	50·4	84·33	4·36	11·31	15·67
			Clover; 1857.				
Aug. 10.	Heads	Unmanured	47·7	85·61	6·00	8·39	14·39
Aug. 10.	Stems and Leaves	Unmanured	59·8	83·23	2·33	14·44	16·77
Aug. 11.	Heads	Unmanured	91·0	87·15	1·89	10·96	12·85
Aug. 11.	Stems and Leaves	Unmanured	42·3	78·32	1·31	20·37	21·68

These experiments also tend to show that the reducing-power of certain of the carbon compounds of the plant was sufficient to convert nearly all the oxygen (or ozone) present into carbonic acid, when in the shade.

The next point is to consider how far the conditions are favourable to the oxidation of Nitrogen in the vegetable organism, when the plant is subjected to the action of the direct rays of the sun.

Experiments, Series 3.

In these experiments, in which over 100 exhaustions were made, the operation was conducted precisely as in the case of the last experiments, with the exception that the plants were exposed during the whole process to the direct rays of the sun. The following Table exhibits a few of the results obtained, which are sufficient for our present purpose.

TABLE III.—Showing the amount and composition of the Gas given off into a Torricellian vacuum, by plants exposed to sunlight.

Date.	How manured, &c.	Total Gas collected, cub. cents.	Per cent.			
			Nitrogen.	Oxygen.	Carbonic acid.	Oxygen and Carbonic acid.
	Wheat (whole plant), 1858.					
June 22.	Unmanured	44·4	73·65	21·17	5·18	26·35
June 23.	Unmanured	34·8	77·01	21·26	1·73	22·99
June 30.	Unmanured	44·1	72·79	20·86	6·35	27·21
June 22.	Mineral and Ammoniacal manure	54·5	73·76	21·29	4·95	26·24
June 23.	Mineral and Ammoniacal manure	42·1	78·15	15·44	6·41	21·85
June 25.	Mineral and Ammoniacal manure	37·2	78·76	19·09	2·15	21·24
	Grass (whole plants), 1857.					
August 15.	Mineral and Ammoniacal manure : second crop	39·0	82·10	16·19	1·71	17·90
August 15.	Mineral and Ammoniacal manure : second crop	47·8	77·08	15·35	7·57	22·92
August 15.	Mineral and Ammoniacal manure : second crop	41·6	76·56	21·46	1·98	23·44
August 17.	Mineral and Ammoniacal manure : second crop	39·9	75·07	23·39	1·54	24·93
August 18.	Mineral and Ammoniacal manure : second crop	36·8	79·88	15·19	4·93	20·12
August 18.	Mineral and Ammoniacal manure : second crop	42·3	80·23	15·97	3·80	19·77
	Beans, 1858.					
July 12.	Mineral manure ; almost podding	44·3	71·11	18·28	10·61	28·89
July 12.	Farm-yard manure ; almost podding	45·8	73·14	10·26	16·60	26·86
July 15.	Unmanured ; almost podding	25·9	82·63	15·83	1·54	17·37
July 15.	Mineral and Ammoniacal manure ; almost podding	30·9	70·55	20·71	8·74	29·45

The general accordance in the proportions of Nitrogen found throughout this Series, together with their general approximation to the amounts observed in Series 2 (Table II.), and the consequent similarity in range of the sums of the two remaining gases—carbonic acid and oxygen—point to the character of the change which has taken place, by virtue of which the proportion of carbonic acid is diminished, and that of oxygen increased. The variations in the amounts are, nevertheless, somewhat considerable ; and we feel that it would be requisite to exercise considerable caution in attempting to refer them to any other than accidental circumstances beyond our control. There can be no doubt, however, that the carbonic acid, shown to exist in the plants in the shade, has yielded the oxygen evolved when in the sunlight. But the mutual relations of the two gases will be more clearly brought to view by a consideration of the results yet to be adduced.

Experiments, Series 4.

These experiments, as well as those of the succeeding Series, were arranged to show the influence of the time of action of the sunlight on the plant, upon the relative proportions of carbonic acid and oxygen.

In the Series of experiments now under consideration, duplicate quantities of the

plant were operated upon at the same time. Both were prepared in the shade; and then the vessels containing them were each entirely excluded from the light, by means of a thick paper covering. In this condition each was attached to a Torricellian exhauster*. The paper was then removed from one of the vessels so as to expose it, with its contents, to the direct rays of the sun; the other vessel, with its enclosed plant, remaining covered. The exhaustion of both was then commenced immediately, and the action continued for half an hour.

The following Table shows the results obtained in this manner, in sunlight, and in the dark, respectively.

TABLE IV.—Showing the amount and composition of the gas evolved, during half an hour, into a Torricellian vacuum, by duplicate quantities of plant, both kept in the dark for some time before commencing the exhaustion, then one exposed to sunlight, and the other kept in the dark, during the process.

(1858.)

Date.	Description of Plant.	Conditions during Exhaustion.	Total Gas collected.	Per cent.			
				Nitrogen.	Oxygen.	Carbonic acid.	Oxygen and Carbonic acid.
			cub. cents.				
	Beans ...	In dark	25·7	66·93	2·33	30·74	33·07
		In sunlight ...	36·4	69·78	8·24	21·98	30·22
July 22.	Oats.....	In dark	28·3	81·63	3·53	14·84	18·37
		In sunlight ...	25·9	70·27	13·13	16·60	29·73
July 23.	Oats.....	In dark	26·4	73·11	8·33	18·56	26·89
		In sunlight ...	22·7	72·25	16·74	11·01	27·75
July 23.	Oats.....	In dark	27·4	68·25	5·11	26·64	31·75
		In sunlight ...	29·2	67·47	19·86	12·67	32·53
July 23.	Oats.....	In dark	31·4	77·39	6·69	15·92	22·61
		In sunlight ...	21·7	76·50	16·59	6·91	23·50

The amounts of carbonic acid and oxygen recorded in the Table, indicate very clearly the ready transformation of the one into the other—or, rather, the transformation of carbonic acid into a solid carbon compound, and free oxygen. In reference to the question we are considering, these results have a high importance, as showing the great reducing-force manifested under the influence of the sun's rays, by which the carbonic acid is so suddenly reduced.

* This term, for convenience, we apply to the apparatus which has been described at p. 487, by which the plant in the vessel, fig. 7, Plate XII., is exhausted.

Experiments, Series 5.

This set of experiments was arranged to show how far the reduction of the carbonic acid, with the evolution of oxygen, was due to the action of the sunlight, in conjunction with the fluids of the plant, at the moment of the passage of the gas through the walls of the cells.

If the decomposition of the carbonic acid resulted from a physico-chemical action, in the presence of sunlight, upon this gas only as it passed through the cell-walls, then there might be no oxygen liberated in the growing cell. If, on the contrary, it were decomposed before passing out of the cell, free oxygen would exist within the latter.

To settle this question, a set of experiments was made exactly similar to those the results of which are given in Table IV., with the exception, that now the time of the exhaustion, and of the action of the sunlight, was reduced to four or five minutes, and the quantity of plant operated upon was increased, so as to give sufficient gas for analysis during this short period. The following Table gives the results obtained.

TABLE V.—Showing the amount and composition of the Gas evolved into a Torricellian vacuum, during four or five minutes only, by duplicate quantities of plant, both kept in the dark for some time before commencing the exhaustion, then one still kept in the dark, and the other exposed to sunlight during the short period of the operation.

(1858.)

Date.	Description of Plant.	Conditions during Exhaustion.	Total Gas collected.	Per cent.			
				Nitrogen.	Oxygen.	Carbonic acid.	Oxygen and Carbonic acid.
			cub. cents.				
July 30.	Oats.....	In dark	41·7	72·42	3·6	23·98	27·58
		In sunlight ...	42·5	72·23	4·71	23·06	27·77
July 30.	Oats.....	In dark	55·7	71·46	3·23	25·31	28·54
		In sunlight ...	43·3	69·98	3·23	26·79	30·02
July 30.	Oats.....	In dark	37·9	83·11	6·86	10·03	16·89
		In sunlight ...	38·5	77·14	9·09	13·77	22·86
July 31.	Oats.....	In dark	34·4	78·49	7·27	14·24	21·51
		In sunlight ...	41·8	75·84	7·89	16·27	24·16

The above results show that the carbonic acid can pass through the cell-wall, in the presence of sunlight, without suffering decomposition. It would hence appear that the free oxygen which a plant yields after it has been for some time under the influence of the direct rays of the sun, existed as such in the cells before the exhaustion. The slight preponderance of oxygen observed in the gas exhausted in sunlight is doubtless due to its action upon the carbonic acid within the cell, during the short period of its operation upon it before it passes out; precisely analogous to the action when the plant is subjected to ordinary atmospheric pressure.

Experiments, Series 6.

In order to bring out more clearly the influence of sunlight before the exhaustion, a series of experiments was made, in which two vessels, containing the duplicate quantities of plant, were each kept covered with paper for some time, and then, from twenty to thirty minutes before commencing the exhaustion, the paper was removed from one of them, both being then exhausted,—the process continuing ten, fifteen, or twenty minutes. The following results were obtained.

TABLE VI.—Showing the amount and composition of the Gas evolved into a Torricellian vacuum, by duplicate quantities of plant, both kept in the dark for some time, and then one exposed to sunlight for about twenty minutes, when both were submitted to exhaustion.

Date.	Description of Plant.	Conditions during Exhaustion.	Total Gas collected.	Per cent.			
				Nitrogen.	Oxygen.	Carbonic acid.	Oxygen and Carbonic acid.
			cub. cents.				
July 31.	Oats.....	In dark	24·0	77·08	3·75	19·17	22·92
		In sunlight ...	34·5	68·69	24·93	6·38	31·31
Aug. 2.	Oats.....	In dark	18·6	68·28	10·21	21·51	31·72
		In sunlight ...	39·2	67·86	25·25	6·89	32·14
Aug. 2.	Oats.....	In dark	30·7	76·87	8·14	14·99	23·13
		In sunlight ...	26·5	69·43	27·17	3·40	30·57
Aug. 2.	Oats.....	In dark	17·0	79·41	7·65	12·94	20·59
		In sunlight ...	28·6	76·22	18·53	5·25	23·78
Aug. 3.	Oats.....	In dark	29·8	81·88	6·38	11·74	18·12
		In sunlight ...	32·1	66·36	30·53	3·11	33·64
Aug. 3.	Oats.....	In dark	11·6	65·52	6·90	27·58	34·48
		In sunlight ...	23·1	70·56	20·35	9·09	29·44
Aug. 3.	Oats.....	In dark	17·0	80·00	5·88	14·12	20·00
		In sunlight ...	19·7	73·10	22·33	4·57	26·90

The comparison of the results in this Table with those in Table V., shows that the oxygen must have been liberated from the carbon, and been retained within the cells, until the instant of the exhaustion, as the gas was evolved from all parts of the leaf, and not from the surrounding water, as soon as the pressure was removed.

The conclusions to be drawn from the above several Series of experiments are not without an interesting bearing upon our present subject.

1. Carbonic acid, within growing vegetable cells, and intercellular passages, which are penetrated by the sun's rays, suffers decomposition with the evolution of oxygen, the latter remaining in the plant or being evolved from it. This takes place very rapidly after the penetration of the sun's rays.

2. Living vegetable cells, &c., which are in the dark, or are not penetrated by the direct rays of the sun, consume the oxygen they contained very rapidly after being placed in such circumstances, carbonic acid being formed.

3. There can hence be little or no oxygen in the living cells of plants during the night, or during cloudy days. The presence of oxygen in the cells of thick-leaved plants, or in the deeper layers of fruit, is also very problematical.

4. With every cloud that passes over the sun, the oxygen of the living cell will oscillate under the influence of the reducing-force of the carbon-matter, forming carbonic acid, on the one hand, and of the reducing-forces of the associated sun's rays, liberating pure oxygen and forming a carbon-compound containing less oxygen than carbonic acid, on the other.

5. The idea is suggested by the above considerations, that there may be in the outer cells, which are penetrated by the sun's rays, a reduction of carbonic acid, and a fixation of carbon, with the evolution of oxygen, at the same time that, in the deeper cells, the converse process of the oxidation of carbon and the formation of carbonic acid is taking place. If such be the case, the oxygen of the outer cells would, according to the laws in conformity with which the diffusion of gases and their passage through tissues are known to take place, be continually penetrating to the deeper cells, and there oxidizing their carbon-matter into carbonic acid; whilst the carbonic acid thus formed would pass in the opposite direction to be decomposed in the sunlight of the outer cells. As the process of cell-formation went forward, and the once outer cells became buried deeper by the still more recent ones above them, they would gradually pass from the state in which the sunlight was the greater reducing-agent, to that in which the carbon-matter of the cell became the greater—from the state in which there was a flow of carbonic acid to them and of oxygen from them, to that in which the reverse action took place. The effect of this action may be the formation of oxidized products—acids, or saccharine matter, &c.—in the deeper cells, whilst the great reducing-power of the sun's rays may form more highly carbonized substances in the outer cells, which in their turn become subject to oxidation when buried deeper. The physical and physiological phenomena of such interchanges are obviously worthy of a closer study; but the subject is too wide for any further development here.

6. The very great reducing-power operating in those parts of the plant where ozone is most likely, if at all, to be evolved, seems unfavourable to the idea of the oxidation of Nitrogen into nitric acid by its means—that is to say, under circumstances where the much more readily oxidizable substance, carbon, is not oxidized, but on the contrary its oxide, carbonic acid, is reduced; whilst, as has been seen, when beyond the influence of the direct rays of the sun, the cells seem to supply an abundance of the more easily oxidized carbon, in a condition of combination readily available for oxidation by free oxygen, or ozone, should it be present. The conclusion that free Nitrogen would not be likely to be oxidated into nitric acid within the structures of the plant, seems to be borne out by the well-known fact, that nitrates are as available a source of Nitrogen

to plants as ammonia; and hence, if we were to admit that Nitrogen can be oxidated into nitric acid in the plant, we must suppose, as in the case of carbon, that there are conditions under which the oxygen compound of Nitrogen is reduced within the organism, and that there are others in which the reverse action, namely, the oxidation of Nitrogen, can take place. In relation to this question, it may be mentioned that several specimens of green Wheat and Grass which had been liberally manured with nitrates were examined for nitric acid, but no trace of it was found in them.

7. To the foregoing six conclusions, another may be here added relating to this subject, though deduced from the results of experiments on the decomposition of organic matter, which will be referred to more fully presently (p. 509 *et seq.*). So great is the reducing power of certain carbon-compounds of vegetable substances, that when the vital (growing) process has ceased, and all the free oxygen in the cells has been consumed, in the formation of carbonic acid, water is decomposed, and hydrogen is evolved. This process does not, however, continue long, showing that the cell provides a certain amount of matter more easily oxidized than the remainder, or that the entire cell-matter, after becoming slightly oxidized, loses its energetic reducing-power. The former alternative is the more probable one.

The foregoing considerations with regard to the intensity of the reducing action of certain of the carbon-compounds in plants suggest the idea of a possible source of Ozone, very analogous to that by which it is ordinarily obtained by means of phosphorus. As is well known, the process consists in allowing oxygen to come into incomplete or only instantaneous contact with phosphorus. This substance having an intense avidity for oxygen, a part of the latter unites with it to form an oxygen-compound of phosphorus, when, if the contact be not too long, another part passes off in the state of Ozone. Certain carbon-compounds of the vegetable cell have also a great affinity for oxygen in the dark (p. 488); and the oscillations of the affinities, due to the degree of light (pages 489–492), and to the depth of the cell (p. 493), would afford conditions of molecular action somewhat similar to those under which Ozone is produced in the presence of phosphorus. According to this analogy the Ozone would be due to the action of the carbon-compounds of the cell on the common oxygen eliminated from carbonic acid by sunlight, and not to the direct action of the sunlight itself. The Ozone thus formed, if not instantly evolved from the plant, would be destroyed by the easily oxidizable carbon-compounds present. It is more probable, however, that the Ozone, stated by De Luca and others to be observable in the vicinity of vegetation, is due to the intense action of the oxygen of the air upon the minute quantities of volatile hydrocarbons emitted by the plants, and to which they owe their peculiar odours, than to any action going on within the cells. The rapidity of the oxidation in the air of the hydrocarbons, and the volatile principles of plants generally, goes to favour the view here suggested; so also does the fact, that Ozone has been observed most readily in the vicinity of such plants as are known to emit freely essential oils—as, for instance, those of the Labiate family.

Since it would appear that, under certain circumstances, Ozone is formed in the immediate vicinity of some plants, it remains to consider the possibility of its acting, in an indirect manner, as a source of combined Nitrogen to our experimental plants—that is, through the agency of the materials involved in the experiment—and thus compromising our result in regard to the question of the appropriation, by the plant itself, of free or uncombined Nitrogen. It might so act :—

1. By becoming absorbed by the water that condenses within the vessel enclosing the plant, and then oxidizing the free Nitrogen dissolved in the water.

2. By being absorbed by the soil—either directly from the air of the enclosing apparatus, or from the condensed water returned to the soil—and then, in connexion with it, as a moist, porous, and alkaline body, forming nitrates in the manner referred to by Pelouze and Fremy*, in their remarks upon the experiments of Cloez which we have shortly described at p. 465 of this paper.

3. By passing down in solution in water, or in the gaseous state, to the older and decomposing parts of the roots, and there forming nitric acid by the oxidation either of the free nitrogen contained in the older cells, or of that evolved in decomposition.

These questions have not been so fully investigated as, considered as independent subjects of inquiry and with reference to the results obtained by Schönbein and others, would be desirable. But so far as they can have a bearing upon the sources of error in our experiments upon the question of the assimilation of free Nitrogen by plants, they have received our careful consideration.

C.—*Experiments on the action of Ozonized air on decomposing Organic matter, and porous and alkaline substances.*

Experiments were made to ascertain the influence of Ozone upon organic matter, and certain porous and alkaline bodies, under various circumstances. The action of ordinary air upon sticks of phosphorus was had recourse to as the source of the Ozone. The arrangement was as follows :—Three large glass balloons (carboys), each of about 40 litres capacity, were connected together by glass tubes which passed through stone-ware stoppers fitted into their mouths, the joints being made tight with calcined gypsum cement. The bottom of each vessel was covered with water to the depth of about half an inch; so that, when pieces of phosphorus were put in, they were partly covered with the fluid. A tube, which could be opened or closed at pleasure, was fixed through each stopper for the supply of water, and fresh phosphorus, as needed. An Allen and Pepys gasometer, capable of holding about 2 cubic feet of air, was connected by a glass tube with the first of the series of vessels; and by its means, air could be forced in a continuous stream through the three vessels containing the phosphorus. On passing out of the last of them it was led through a wash-bottle, and then into a glass vessel, from which, by means of a number of glass tubes passing from it, it was distributed into bottles containing the substances to be submitted to the action

* Traité de Chimie Générale, tome sixième, p. 343 (1857).

of the Ozone. Thus, all the ozonized air passed the wash-bottle in its course from the balloons to the distributing apparatus.

The following substances were subjected to the action of the Ozone—each substance, or mixture, being enclosed in a glass bottle of about 1·5 litre capacity, fitted with an exit-tube in which were fragments of pumice saturated with sulphuric acid :—

(1) ¾ lb. of ignited soil, moistened with 100 cub. centims. water, this being just sufficient to make it slightly coherent.

(2) ¾ lb. of ignited soil, 300 cub. centims. water, 2·5 ounces boiled starch, and 2·5 ounces dry starch.

(3) ¾ lb. of ignited soil, 200 cub. centims. water, and 2·5 ounces saw-dust.

(4) 2·5 ounces saw-dust, and 100 cub. centims. water.

(5) ¾ lb. of ignited soil, 200 cub. centims. water, and 2·5 ounces bean-meal.

(6) ¾ lb. of ignited soil, 150 cub. centims. water, and 2·5 ounces bean-meal.

(7) 2·5 ounces bean-meal, and 50 cub. centims. water.

(8) 1 lb. garden soil.

(9) ¾ lb. of slaked lime, and 2·5 ounces bean-meal, made slightly pasty with water.

(10) ¾ lb. of slaked lime, some starch, and saw-dust, made slightly pasty with water.

(11) 2·5 ounces of boiled starch, 2·5 ounces fresh starch, and 200 cub. centims. water.

All the bottles were placed before a window where the sun shone directly upon them for a considerable part of the day, as it did also for some hours daily upon the balloons.

Every day, about 9 o'clock in the morning, the cylinder of the gasometer was raised, and a slow current of air passed through the apparatus during about two hours. This process was generally repeated once or twice more during the day. The experiment commenced in April, and continued till the following autumn ; that is, through all the warm weather of the summer, when a thermometer in the room frequently stood at 25° to 29° C. The amount of Ozone passing through the apparatus was so great, that the vulcanized caoutchouc which connected the tube from the last balloon with that passing into the wash-bottle was cut off with the passage of three or four gasometerfuls of air. The joint was then made by fixing a piece of larger glass tubing over the point of contact of the smaller connecting tubes, and closing the ends of the larger tube with corks well fitted upon the smaller ones.

Once every three or four days a small piece of phosphorus was dropped into each balloon. In this way the action was sufficiently maintained to produce a distinct odour of Ozone in the room whilst the air was passing.

During the first half of the period of the experiment, a wash-bottle filled with large lumps of pumice, and about half-full of a solution of caustic potash, was used ; so that the ozonized air in bubbling rapidly through the solution continually threw it up, by which means the pumice was kept moistened with it.

A careful examination of this liquid, together with the washings of the pumice, failed to detect any nitric acid. About the 1st of July, the alkaline wash was replaced by a

bottle containing only pure water. The latter also, at the termination of the experiment, failed to give evidence of even traces of nitric acid.

At the termination of the experiment, the contents of each of the eleven bottles were also examined. A portion was exhausted with water, and the extract concentrated by boiling, after the addition of permanganate of potash to destroy the organic matter. The excess of permanganic acid was removed by carbonate of lead, and the clear solution filtered off from the precipitate. Each solution so obtained was tested for nitric acid ; but in no case, excepting that of the garden soil, was there any indication of its presence. An examination of the original garden soil showed that it contained nitric acid before being subjected to the action of the Ozone.

Owing to the negative character of the above results, it is not necessary to describe the apparatus, and the circumstances of the experiments, in any more detail, which would have been desirable had the results been of a positive kind.

We are, however, by no means prepared to infer, from the evidence just adduced, that under no circumstances in nature is it possible for Ozone to transform nitrogenous compounds of the ammonia class, or the nascent nitrogen evolved during decomposition, into oxides of Nitrogen. We would not say that it may not be possible for Ozone to form such compounds when in connexion with non-nitrogenous bodies or porous substances permeated with gaseous Nitrogen, or even in the atmosphere. Nor are we prepared to maintain that the nitric acid in soils is not in part due to some of these causes. These questions will require much further investigation before they can be satisfactorily settled. To some of them we shall refer again presently.

But we wish particularly to call attention to the fact that, in the experiments just referred to, there was a very much larger quantity of Ozone, acting upon organic matter, soil, &c., in a very wide range of circumstances, and for a much longer period of time, than was involved in our experiments on the question of the assimilation of free Nitrogen by plants. Yet there was no appreciable quantity of nitric acid formed. It may therefore be concluded that there will be no error introduced into the results of the experiments on the question of the assimilation of free Nitrogen by plants themselves, arising from the action of Ozone upon free Nitrogen under the circumstances of the experiments, and so providing to the plants an unaccounted supply of combined Nitrogen.

D.—*Evolution of free Nitrogen in the decomposition of Nitrogenous organic compounds.*

Two obvious methods of investigating the question, whether or not free Nitrogen is given off in the decomposition of nitrogenous organic matter, present themselves.

1. To allow the organic matter to decompose under circumstances in which any free Nitrogen that may be evolved can be collected and estimated.

2. To allow the organic matter to decompose under circumstances in which the total amount of the compounds of Nitrogen formed in the process can be estimated—the loss of Nitrogen then representing the free Nitrogen evolved.

A number of experiments according to the first of these methods has been made by Reiset. He submitted nitrogenous animal and vegetable substances to decomposition under an enclosing vessel in ordinary air, into which he passed oxygen as that of the air was consumed. His result was, that the amount of Nitrogen in the air was gradually increased. He does not appear, however, to have completed the inquiries on this subject which he proposed to undertake.

The second method has been followed by M. G. Ville. The conclusion he arrived at was, that in the decomposition of several nitrogenous vegetable substances, about one-third of their total Nitrogen was evolved in the free state.

The losses of Nitrogen which M. Boussingault's experiments on the question of the assimilation of free Nitrogen by plants indicated, when he used nitrogenous organic matter as manure, rendered it desirable to investigate the subject in its bearings upon the conditions provided in our own experiments on that question. The following plan was adopted :—

A given weight of nitrogenous organic matter, the percentage of Nitrogen in which had been previously determined, was mixed with burnt soil, or pumice, prepared as for the experiments on the assimilation of Nitrogen by plants (p. 471), and put into a bottle of about 360 cub. centims. capacity, as shown at B, fig. 8, Plate XII. A proper quantity of water was added ; and then the bottle was closed with a cork, through which were tightly fitted two bent glass tubes, which passed externally in opposite directions. One of these tubes was connected with an eight-bulbed apparatus A, containing sulphuric acid, for the purpose of washing air drawn through it into the rest of the apparatus. The other tube, passing from B in the opposite direction, was connected with a similar eight-bulbed apparatus C, containing a solution of oxalic acid. From this again passed a tube extending, through a cork to the bottom of the second bottle D (similar to B), which contained some sulphuric acid. Through the cork of the bottle D another tube E also passed, but it did not dip into the sulphuric acid. It is obvious that, on drawing the air from D by means of the tube E. a current of air would pass inwards through the sulphuric acid in A, into the bottle B, then through the oxalic acid in C. and so on. In this way, the air of the vessel B, containing the decomposing organic matter, could be renewed at pleasure by fresh air, washed free from ammonia. At the same time, any ammonia evolved from the decomposing organic matter was drawn into the eight-bulbed apparatus C, and there absorbed by the oxalic acid. At the termination of the experiment, the combined Nitrogen remaining in B, and that retained in the form of ammonia in the oxalic acid in C, were determined. The difference between the total amount of combined Nitrogen so found in the products and that originally contained in the organic substance submitted to decomposition, is taken to represent the amount of nitrogen given off, in the free state, during the process.

SERIES 1.—*Experiments on the decomposition of nitrogenous organic matter, made in* 1857.

Wheat-meal, Barley-meal, and Bean-meal were the nitrogenous organic substances submitted to decomposition. A quantity of each of these was mixed respectively with burnt soil and with pumice, making in all six separate experiments. About 100 grammes of soil, or about 60 grammes of pumice, were used—these quantities, together with the meal, filling the bottles B to the depth of about 2 inches. Sufficient water was added to bring the mixture into an agglutinated condition. The materials being so prepared, the apparatus was put together according to the arrangement above described. The six sets were then placed in a light room before a large window, so that, during the middle of the day, the sun shone directly upon them.

The experiments commenced on June 10, and terminated on October 8, 1857. Several litres of air were drawn through each apparatus daily, by applying the mouth to the tube E. After the first day the gas possessed a more or less disagreeable taste, and the odour of decomposing organic matter.

The following statement of the condition of the several mixtures, at the termination of the experiment, is condensed from the notes then made :—

1. *Wheat-meal and ignited Pumice.*—The meal slightly mouldy; the odour that of decomposing organic matter; quite moist, so that the particles of pumice adhered together.

2. *Wheat-meal and ignited Soil.*—A slight mouldy coating on the surface; odour like that of No. 1; the mass moist, but not sufficiently so for the particles of soil to agglutinate.

3. *Barley-meal and ignited Pumice.*—No mouldy coating on the surface; odour similar to that of the wheat but more intense, and sour, much like that of fermenting malt; the mass wet and clammy.

4. *Barley-meal and ignited Soil.*—No mouldy coating on the surface; odour like that of barley (No. 3); sufficiently moist to agglutinate.

5. *Bean-meal and ignited Pumice.*—A little mould upon the surface, but not quite so much as with the wheat and soil (No. 2); odour very disagreeable and putrescent; the mass wet and clammy.

6. *Bean-meal and ignited Soil.*—Very similar to the bean-meal and ignited pumice (No. 5), but a little more wet and pasty.

In every case, carbonic acid was evolved on the addition of oxalic acid, preparatory to evaporating to dryness. The evolution was the greatest from the bean-meal with soil.

A known proportion, about one-half, of each dried mass, was burnt with soda-lime, and the Nitrogen capable of estimation in that way determined. The remainder was reserved for the determination of nitrates, provided any were present. On examination, however, no nitric acid was detected. To put the validity of the qualitative test for nitric acid beyond doubt, 0·001 gramme of nitric acid was added to the portion of substance which had been already exhausted to test for nitric acid, and had yielded a nega-

tive result. The mass was then re-exhausted with water, and the extract submitted to precisely the same process as before, when the presence of nitric acid was made manifest.

In the following Table are given the numerical results of the six experiments :—

TABLE VII.—Showing the Numerical results of experiments on the Decomposition of Nitrogenous organic matter, made in 1857.

	Substances submitted to experiment.				Nitrogen after Decomposition		
	Description of Organic matter.	Description of Matrix.	Quantity of "Meal" taken.	Quantity of Nitrogen.	Total by Soda-lime.	Not recovered.	
						Actual quantity.	Per cent.
			grammes.	grammes.	grammes.	grammes.	
1.	Wheat-meal	Ignited pumice ...	2·0585	0·0370	0·0338	0·0032	8·51
2.	Wheat-meal	Ignited soil.....	2·1282	0·0383	0·0335	0·0048	12·53
3.	Barley-meal	Ignited pumice ...	2·2495	0·0380	0·0368	0·0012	3·16
4.	Barley-meal	Ignited soil.....	2·0980	0·0355	0·0309	0·0046	12·96
5.	Beau-meal ...	Ignited pumice ...	2·0650	0·0803	0·0741	0·0062	7·72
6.	Bean-meal ...	Ignited soil.....	2·0800	0·0809	0·0823	(+0 0014)	+ (1·73)

The last two columns of this Table, which exhibit respectively the actual amount of Nitrogen not recoverable by the soda-lime process in the substance after decomposition, and the percentage proportion of this loss upon the Nitrogen submitted to experiment, are the most important to consider for our present purpose.

With one exception (the gain of Nitrogen in which is quite within the range of the error of analysis), all the experiments point to the fact, that a part of the Nitrogen of decomposing organic matter passes into a state in which it cannot be estimated by the soda-lime process. Neither did it exist as nitric acid. There appears, therefore, to be an evolution of free Nitrogen.

It is not a little remarkable, that although so large a proportion of the total Nitrogen present is lost, doubtless passing off as free Nitrogen, yet scarcely a trace of ammonia was given off from the mass ; for the oxalic acid in the bulb-apparatus C was, in each case, separately rendered alkaline with caustic potash and distilled, the distillate being collected and examined quantitatively for ammonia, when, in only one case—that of the Bean-meal and Pumice—was there any ammonia indicated, and then only equal in amount to 0·0002 gramme Nitrogen. This was the case, notwithstanding that the Nitrogen in the mixtures amounted to from 0·03 to 0·08 per cent. of their entire quantity.

The questions here arise:—to what extent had the decomposition of the organic substance proceeded? what shall we accept as the measure of the amount of decomposition? what are the intermediate stages through which the substance has passed? what is the character of the organic compounds remaining in the mass? what is the nature of those that have been evolved? and what part does water play in the matter?

The subject of the character of the gradual changes which take place during the

decomposition of mixtures of nitrogenous and non-nitrogenous substances, in variable proportions, in connexion with soil and water, involves points so highly complicated, that we cannot pretend satisfactorily to answer all the above questions.

We may, however, ascertain the character of some of the final products of the decomposition, and from a knowledge of these draw conclusions as to the changes of which they are the result under various circumstances.

Series II.—*Experiments on the Decomposition of nitrogenous Organic Matter, made in* 1858.

The following series of experiments was made with a view to embrace a wider range of conditions as to degree of moisture;—to observe the different stages of decomposition as manifested by the odour, &c.;—to include the circumstances of sprouting, early growth, and subsequent decay of the products of the vegetation;—and to afford material for a more elaborate inquiry into the character of the products of the decomposition.

The results given above, in Table VII., do not show any difference between soil and pumice as matrix that we can safely refer to other than incidental causes independent of the action of the matrix itself. Yet we continue the use of the two substances, in order to see if, with a larger percentage of organic matter, and a more complete decomposition, the pumice will retain the ammonia formed as well as the soil.

About 175 to 200 grammes of soil, or 120 to 150 grammes of pumice, were used as matrix in each experiment, and the other conditions were as follow :—

Wheat.	*a.*	171 seeds, weighing 8·0475 grammes,	50 c. c. water,	with ignited Soil.
	b.	171 seeds, weighing 8·0715 grammes,	100 c. c. water,	with ignited Pumice.
	c.	Meal, weighing 9·8810 grammes,	40 c. c. water,	with ignited Soil.
Barley.	*a.*	163 seeds, weighing 8·0440 grammes,	50 c. c. water,	with ignited Soil.
	b.	163 seeds, weighing 8·1360 grammes,	100 c. c. water,	with ignited Pumice.
	c.	Meal, weighing 8·9670 grammes,	40 c. c. water,	with ignited Soil.
Bean.	*a.*	7 seeds, weighing 6·4700 grammes,	50 c. c. water,	with ignited Soil.
	b.	7 seeds, weighing 5·7830 grammes,	50 c. c. water,	with ignited Pumice.
	c.	Meal, weighing 6·1750 grammes,	40 c. c. water,	with ignited Soil.

Those of the mixtures to which about 50 cub. cent. of water were added, were about as moist as soils when in a good condition for vegetable growth. Those with 40 cub. cent. were much drier in appearance, there being no tendency to agglutination of the particles. Those with 100 cub. cent. were very wet, there being some free water above the solid matters.

The seeds sown with 50 cub. cent. water showed growth in a few days after being put in, and the vessels (B, fig. 8, Plate XII.) were soon filled with a mass of vegetation. Those sown with double this quantity, or 100 cub. cent. water, showed no indications of sprouting; and in a few days, the odour evolved from them showed that decomposition had set in. The mixtures of meal and soil, also, soon gave odours indicative of

decomposition, though less foul than those from the whole seed and 100 cub. cent. water.

The following Notes, taken at different times during the experiments, will indicate the stages of growth, or decomposition, through which the several organic matters passed.

March 16.

Wheat (*a*)—*Seeds, in Soil with* 50 *cub. cent. water.*—Came up some days later than the corresponding Barley *a*; has not grown so rapidly; has kept green for a longer period and is yet growing healthily, though much crowded in the small bottle. The air passing from the bottle has not the odour of decomposing organic matter. There is a slight mould on the soil due to a few seeds which did not grow.

Wheat (*b*)—*Seeds, in Pumice with* 100 *cub. cent. water.*—The Pumice in this case was covered with water to the depth of about one-fourth of an inch, and a few grains floated in the water. In a few days the air drawn through the bottle gave the odour and taste of decomposing organic matter. At the end of about a month the free water on the surface began to disappear rapidly, and in a short time it was all gone, leaving a grey mouldy coating of organic matter over the top of the pumice. This disappearance of water was too great to be due to simple evaporation in the air passed through the apparatus. It was doubtless consumed in the process of decomposition—a view which receives confirmation from our experiments on the nature of the gases evolved during decomposition.

Wheat (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—Gives little indication of decomposition by the air which passes from it. Compared with Wheat *b*, the difference in this respect is very marked.

Barley (*a*)—*Seeds, in Soil with* 50 *cub. cent. water.*—Came up soon after being put in, grew rapidly, and in five weeks had grown to the top of the bottle, a height of about 5 inches. By the end of February the bottle was quite filled with green vegetable matter, and up to that time no odour of decomposition was distinguishable in the air which was passed through, but from that date the leaves became yellow, and decomposition has been manifested both by appearance and the taste of the air.

Barley (*b*)—*Seeds, in Pumice with* 100 *cub. cent. water.*—Progress almost exactly similar to that of the corresponding Wheat (*b*) described above.

Barley (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—Very like the corresponding Wheat (*c*) above.

Bean (*a*)—*Seeds, in Soil with* 50 *cub. cent. water.*—Came up a week after sowing. The sprouts pushed several seeds out of the soil, yet they have continued to grow up to the present time, lying upon the surface. At first there was a natural development of leaf and of roots; but soon the latter took a remarkable course, coming through the surface of the soil and extending through all parts of the bottle, mingling with the

stems and leaves, and forming a densely crowded mass of vegetable matter. The rootlets from the main branches extending through the mass commenced their growth in all directions indiscriminately, but after growing about one-fourth of an inch they invariably turned downwards.

Bean (*b*)—*Seeds, in Pumice with* 50 *cub. cent. water.*—Identical in appearance with the last (Bean *a*), excepting a little further developed.

Bean (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—Almost exactly like the Wheat (*c*) and Barley (*c*) meals, described above.

In no one of the above nine cases was there any Ozone reaction to test-paper.

April 28.

Wheat (*a*)—*Seeds, in Soil with* 50 *cub. cent. water.*—Twelve to fifteen stems; leaves not unrolled, and scarcely any tendency to expansion. The vegetation not nearly so much crowded as in the case of the corresponding Barley (*a*); yet most of the shoots show signs of dying. A thin coat of fungoid growth covers the stems to the height of from 1 to 1·5 inch. The stems are from 2 to 2·5 inches high, those of the corresponding Barley being from 3 to 4 and 5 inches high. The air passed through the apparatus is not disagreeable either in taste or odour.

Wheat (*b*)—*Seeds, in Pumice with* 100 *cub. cent. water.*—The Pumice moist, but without visible water, and the surface covered with a grey mouldy coating. The air has had an unpleasant odour ever since March 16, and now it is exceedingly nauseating.

Wheat (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—The soil apparently dry, but slightly mouldy, and the air passed over is almost without odour.

Barley (*a*)—*Seeds, in Soil with* 50 *cub. cent. water.*—The bottle full of vegetable matter, all quite yellow at the top where it touches the cork, and yellowish lower down. The plants covered with a coating of greyish fungus. The odour and taste of the air slightly disagreeable. The soil looks quite dry.

Barley (*b*)—*Seeds, in Pumice with* 100 *cub. cent. water.*—The soil is moist and mouldy. The mould on the surface appears to be decreasing, and is now less abundant than in the case of the corresponding Wheat (*b*). The odour of the air is much less disagreeable; indeed there is scarcely any at all.

Barley (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—The soil mouldy and apparently dry. The air from the vessel tasteless, and inodorous.

Bean (*a*)—*Seeds, in Soil with* 50 *cub. cent. water.*—Continued to grow vigorously for a long time, filling the bottle with a confused mass of stems, leaves, and roots, which has commenced to decay rapidly during the last two weeks. The upper portions of the mass are now entirely dead and black; but nearer the soil the stems and leaves are green and long, whilst healthy roots are intermingled with them. The soil is also tolerably filled with roots. The odour of the air is not disagreeable.

Bean (*b*)—*Seeds, in Pumice with* 50 *cub. cent. water.*—Very much like the last (Bean *a* with soil), excepting that the development of roots is scarcely so great.

Bean (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—A little mouldy matter on the surface of the soil, which appears dry.

July 1.

Wheat (*a*)—*Seeds, in Soil with* 50 *cub. cent. water.*—Plants all dead; the entire contents of the bottle apparently quite dry. The air has a slight musty odour.

Wheat (*b*)—*Seeds, in Pumice with* 100 *cub. cent. water.*—Odour rather more marked than that of the last (Wheat *a*) ; a coating of organic matter on the surface of the pumice.

Wheat (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—Soil quite dry ; covered with mould ; odour of air slight.

Barley (*a*)—*Seeds, in Soil with* 50 *cub. cent. water.*—Plants quite dead and dry ; air inodorous.

Barley (*b*)—*Seeds, in Pumice with* 100 *cub. cent. water.*—Soil dry and covered with mould. Air like that of Wheat *b* ; more foul than that of any of the others.

Barley (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—Surface dry. The air has a slightly musty odour.

Bean (*a*)—*Seeds, in Soil with* 50 *cub. cent. water.*—Plants all dead, and much decom posed ; forming a black mouldy mass of organic matter on the surface of the apparently dry soil. The air has no perceptible odour.

Bean (*b*)—*Seeds, in Pumice with* 50 *cub. cent. water.*—The same as the last (Bean *a*).

Bean (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—Soil dry ; slightly mouldy ; the air from over it inodorous.

In order to see the effect upon the organic matter of an increased amount of moisture, 100 cub. cent. of water were added to each of the nine bottles of decomposing matter at this date (July 1).

August 28.

Final Report, and termination of the Experiment.

Wheat (*a*)—*Seeds, in Soil with* 50 *cub. cent. water.*—Very little odour, and that not unpleasant. On removal from the bottle, it was found that the organic matter was well decomposed, only very indefinite remains of stems and leaves being visible in the soil. On the addition of oxalic acid to the mass, to retain the ammonia during the evaporation to dryness, a copious evolution of carbonic acid took place, and the surface of the fluid was constantly covered with a brown froth during the process.

Wheat (*b*)—*Seeds, in Pumice with* 100 *cub. cent. water.*—The mass has a disgusting mouldy odour. The form of the grain is retained, but all the contents are gone, and the

husk is filled with fluid. On evaporation with oxalic acid, there was evolution of carbonic acid, &c., as with the last; indeed it was the same with all those which follow.

Wheat (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—In this, as in all the other cases, owing to the water added on the 1st of July, the mass was covered to the depth of from $\frac{1}{4}$ to $\frac{1}{2}$ an inch with fluid. In both the above cases with Wheat, the supernatant water was colourless, but in this it had a dirty, muddy, yellowish colour. The mass emitted a foul disagreeable odour, though not so intense as that of the corresponding Barley.

Barley (*a*)—*Seeds, in Soil with* 50 *cub. cent. water.*—The organic matter thoroughly decomposed; stems, roots, and leaves no longer distinguishable in the soil; other conditions about as those with the corresponding Wheat *a*.

Barley (*b*)—*Seeds, in Pumice with* 100 *cub. cent. water.*—The pumice covered with a black coating of organic matter; supernatant water clear. The odour of the air above the mixture exceedingly disgusting, resembling that of decaying excrements; traces of sulphide of hydrogen perceptible. The form of the seeds is preserved, but the shell contains only fluid.

Barley (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—Supernatant water yellowish; odour musty, but not very disagreeable. Decomposition so complete that traces of organic matter are hardly perceptible.

Bean (*a*)—*Seed, in Soil with* 50 *cub. cent. water.*—The organic matter well decomposed. Odour musty.

Bean (*b*)—*Seeds, in Pumice with* 50 *cub. cent. water.*—Plants well decomposed; only very indefinite skeletons of stems, leaves, and roots remaining. Odour musty, but not disagreeable.

Bean (*c*)—*Meal, in Soil with* 40 *cub. cent. water.*—Supernatant water slightly yellow. Odour musty, but not offensive.

The last description, dated August 28, refers to the state of the respective masses just before being dried for analysis. After drying, any slight remains of organic matter had become brittle; and the substance, in every case excepting where 100 cub. cent. water had been added at the commencement, presented the appearance of clean soil or pumice, without organic matter. In the excepted cases the shell of the grain was still visible. If we take into consideration the amount of growth in several of the cases on April 28, it will be seen how great must have been the subsequent decomposition so entirely to get rid of the organic matter.

It is worthy of remark, that, in a few instances, the sulphuric acid in the bottle D, fig. 8, Plate I., became coloured slightly brown, indicating the passage into it, through the oxalic acid, of some carbon-compound more complicated than carbonic acid. In the course of other parts of our investigation, we have observed phenomena indicative of a similar result; but as we have not followed up the subject, we leave it with only this remark as to the fact of what we have observed.

The following Tables (VIII. and IX.) show the numerical results of the investigation now under consideration :—

TABLE VIII.—Showing the conditions provided in Experiments on the Decomposition of Nitrogenous Organic Matter ; and the amount and proportion of the original *Carbon* of the substance remaining after the decomposition, or given off during the process.

Substances involved in the Experiment.				Weight of Organic Matter.		Carbon in Organic Matter.			
Organic Matter.		Matrix.	Water.	Fresh.	Dry.	Before Decomposition.	After Decomposition.	Loss in Decomposition.	
Description.	Condition.							Actual quantity	Per cent.
			cub. cent.	grammes	grammes	grammes	grammes	grammes	
1. Wheat	*a.* 171 seeds	Ignited soil	50	8·0475	6·7438	3·1089	0·9274	2·1815	70·17
	b. 171 seeds	Ignited pumice	100	8·0715	6·7639	3·1182	0·9178	2·2004	70·56
	c. Meal	Ignited soil	40	9·8810	8·2803	3·8172	1·3199	2·4973	65·42
2. Barley	*a.* 163 seeds	Ignited soil	50	8·0440	6·7127	3·0523	0·9598	2·0925	68·55
	b. 163 seeds	Ignited pumice	100	8·1360	6·7895	3·0872	1·1952	1·8920	61·28
	c. Meal	Ignited soil	40	8·9671	7·4830	3·4025	1·0995	2·3030	67·68
3. Beans	*a.* 7 seeds	Ignited soil	50	5·7830	4·5830	2·2915	0·8511	1·4404	62·86
	b. 7 seeds	Ignited pumice	50	6·4700	5·1275	2·5637	...	...	...
	c. Meal	Ignited soil	40	6·1750	4·8937	2·4468	0·9778	1·4690	60·04

TABLE IX.—Showing the conditions provided in Experiments on the Decomposition of Nitrogenous Organic Matter ; the amount and proportion of the original *Nitrogen* remaining after the decomposition, or given off during the process ; together with the amount *evolved* as *Ammonia*, or remaining in the products *as such*.

Substances involved in the Experiment.				Total Nitrogen in Organic Matter.				Nitrogen in the form of Ammonia.			
Organic Matter.		Matrix.	Water.	Before Decomposition.	After Decomposition.	Loss (or Gain).		Total quantity.	Per cent.	Absorbed by Oxalic acid during Decomposition.	
Description.	Condition.					Actual quantity	Per cent.			Actual quantity.	Per cent.
			cub. cent.	grammes	grammes	grammes		grammes		grammes	
1. Wheat	*a.* 171 seeds	Ignited soil	50	0·1392	0·1398	+ 0·0006	+ 0·43	0·0429	30·83	·00038	0·273
	b. 171 seeds	Ignited pumice	100	0·1396	0·1214	0·0182	13·08	0·0573	41·06	·00002	0·014
	c. Meal	Ignited soil	40	0·1709	0·1680	0·0029	1·74	0·0197	11·49	·00040	0·234
2. Barley	*a.* 163 seeds	Ignited soil	50	0·1247	0·0746	0·0501	40·20	0·0157	12·64	·00055	0·441
	b. 163 seeds	Ignited pumice	100	0·1261	0·1052	0·0209	16·62	0·0294	23·39	·00002	0·016
	c. Meal	Ignited soil	40	0·1390	0·1311	0·0079	5·66	0·0166	11·97	·00039	0·280
3. Beans	*a.* 7 seeds	Ignited soil	50	0·2417	0·2107	0·0310	12·84	0·0140	57·91	·00341	1·424
	b. 7 seeds	Ignited pumice	50	0·2704	0·2380	0·0324	11·99	.....	.....	·00242	0·895
	c. Meal	Ignited soil	40	0·2581	0·2267	0·0314	12·16	0·1039	40·25	·00060	0·232

A comparison of the results in Tables VII. and IX. will show that they are confirmatory of each other as to their more general indications. Both series agree in the entire absence of any tangible relation between the varied circumstances of decomposition, and the products of that decomposition.

It is quite evident that, whilst in some instances there has been no evolution of Nitrogen, in others the amount of decomposition involving such evolution has been very great. Indeed, in some cases, the indication of the loss of Nitrogen in this way is so great that we could not have believed such a result possible had it not been attested by repeated analysis. But we have not been able to trace these differences to their ultimate causes.

The amount of decomposition, as indicated by the physical condition of the several substances at the termination of the experiment, as also by the proportion of carbon given off as shown in Table VIII., might lead to the conclusion that the process had gone about equally far, and attained about an equal completeness, in all the cases to which Tables VIII. and IX. refer. But here the equality of effect ceases. Thus, from 60 to 70 per cent. of the total carbon in the original organic matter has passed off; but the proportion of the original Nitrogen that is not recovered in the products varies, under the same circumstances, from 0 to 40 per cent. of it. The proportion of the Nitrogen of the original substance which was retained in the mass, or absorbed in the oxalic acid in the bulb-apparatus (C) in such form as to be given off as ammonia on distillation from a weak alkaline solution, and which probably existed, therefore, in the products as ammonia, ranged from 12 to 58 per cent. of the total quantity involved in the experiment. And, again, the proportion of the Nitrogen evolved from the mass as ammonia during the decomposition, and which was retained in the oxalic acid solution (C), varied from 0 to about 1·5 per cent. of the original or total Nitrogen.

If we attempt to trace a relation between the loss of carbon, the loss of nitrogen, the formation of ammonia, and the evolution of the small amounts of it during the decomposition, on the one hand, and the circumstances of matrix, moisture, growth, decay, &c., pointed out in the notes preceding the Tables, we fail to discover any connexion which we may with safety regard as exhibiting cause and consequence.

The most that we can venture to say is that, under a wide range of circumstances, a considerable loss of Nitrogen occurs in the decomposition of nitrogenous organic matter; that under particular, and apparently rather rare circumstances, this loss of Nitrogen does not occur; that the proportion of the Nitrogen taking, under the same circumstances, such form that it may be driven off as ammonia on the distillation of the products with a weak solution of alkali, varied from one-eighth to more than one-half of the total present; and that the amount of the Nitrogen evolved from the mass as ammonia during the process was quite inconsiderable.

These conclusions, though necessarily expressed in very general terms, have nevertheless a very important bearing on certain questions in practical agriculture. Whilst it would appear that there may be a very great loss of Nitrogen—a very important element in manure—under circumstances of decomposition of organic matter, closely allied to those to which, in practice, nitrogenous organic manures are subject, it is at the same time indicated that it is possible for such matters to pass through the process of decomposition without such loss. The importance of further investigation is hence

suggested, to ascertain the causes of the difference of effect, in order, if possible, to control them. The results also point to the insignificance of the loss of Nitrogen in the form of ammonia, a supposed evil to which the attention of agricultural chemists has specially been directed in order to find means of preventing it, though nothing has as yet been done to avoid the loss, in apparently much larger quantity, of free Nitrogen. But as these questions are more appropriate for consideration in a purely agricultural paper, we shall not follow them further in this place.

Other investigations, to which we have to call attention, will throw some light upon the character of the molecular forces by which the decomposition of nitrogenous organic compounds is effected under such circumstances as we have been considering. These forces might be one or both of two kinds.

1. They might be of an oxidizing character, analogous to that of the action of chlorine upon ammonia by which free Nitrogen is evolved.

2. They might be of a reducing character, similar to that of a great number of substances upon the oxygen-compounds of Nitrogen, by which the oxygen of the latter is appropriated, and free Nitrogen given off.

3. These two actions may operate in succession the one to the other.

It is well known that an oxidizing action may be so intense as to deprive a nitrogenous organic compound of all its carbon and hydrogen, converting it into oxygen compounds, as is done by permanganic acid. The converse action of the transformation of oxygen-compounds of Nitrogen into ammonia is also very well known. An intermediate stage in either of these converse actions may give free Nitrogen.

There can be little doubt that the Nitrogen in the organic substances which we have submitted to decomposition existed in them in a condition more analogous to a hydrogen than to an oxygen compound of it. The able researches of Hofmann into the nature of compounds formed upon the ammonia type, would lead us to suppose that the Nitrogen compounds upon which we have been operating are of the ammonia class. They are more difficult to oxidize into nitric acid than is ammonia; yet their transition into ammonia is so easy, that it is effected in almost all the chemical changes to which they are ordinarily subjected. And, since ammonias yield free Nitrogen under the influence of oxidizing forces, it may be inferred that it has been under the influence of such forces that Nitrogen has been set free in the cases recorded above. Pelouze has remarked * that salts of nitric acid are converted into ammonia, in contact with decomposing organic matter. Experiments of our own have shown that, during the decomposition of organic matters in contact with nitrates, free Nitrogen is not evolved. The evolution of free Nitrogen in the experiments quoted above must, therefore, be referred to the action of oxidizing forces.

The experiments next referred to bear upon these points.

* Comptes Rendus, xliv. p. 118.

E.—*Experiments on the action of the oxidizing and reducing forces, as manifested in the decomposition of organic matters containing Nitrogen.*

Several qualitative experiments showed that when cereal grains and leguminous seeds were placed in water, over mercury, an evolution of gas took place, after about thirty-six to forty-eight hours. This went on rapidly for a week or two, after which all action appeared to cease, no more gas being evolved. The total quantity of gas evolved varied between 20 and 50 cub. cent. from 3 to 4 grammes of the seeds. An examination of the gas proved it to be almost entirely carbonic acid and hydrogen, the quantity of Nitrogen being very small.

To examine this action more thoroughly, about half a pound of a mixture of Wheat, Barley, and Beans was taken, put into a long narrow glass vessel (fig. 7, Plate XII.) of about 500 cub. cent. capacity, which was then filled with well-boiled water, and closed with a cork, through which two glass tubes (*a* and *b*) passed. The external ends of these tubes were fitted with caoutchouc tubing, for closing with pinch-cocks, or connexion with the Torricellian exhauster as described at p. 487. One of the tubes being so connected with the exhauster, it was allowed so to remain for several hours, in order to remove all the gaseous Nitrogen from the seeds. The vessel was then inverted in mercury, with one of the tubes (*b*) open under that fluid, and the whole placed in sunlight to favour the decomposition. This was done on the 28th of August, 1858. The seeds commenced swelling very soon, and on the 30th of August well-marked decomposition had set in.

On September 13th the vessel was about two-thirds full of gas, the displaced water having passed out through the quicksilver. Part of the seed was now above the water, in the gas, which commenced bubbling out through the tube (*b*). The arrangement was allowed so to remain until October 5, when 400 cub. cent. of gas were collected, of which the percentage composition was as follows :—

	Carbonic acid.	Hydrogen.	Nitrogen.
Experiment 1	64·87	34·83	0·30
Experiment 2	64·54	35·46	traces.

The quantity of the gas evolved points to the extent of the decomposition ; the amount of carbonic acid and hydrogen shows how great must have been the reducing force exerted ; and the small quantity of Nitrogen, which was probably due to accident, indicates that free Nitrogen was not a product of the action.

The vessel was again filled with boiled water, again connected for some time with the Torricellian exhauster, and again placed in its former position in the sunlight.

October 9.—A small bubble of gas collected in the top of the vessel.

November 3.—Only a few bubbles of gas at the top of the vessel.

November 17.—The vessel was removed into the laboratory and placed in a room, the temperature of which varied from a few degrees above the freezing-point to about 24° C.

December 1.—Very little gas evolved.

December 12.—The gas collected without exhaustion measured only 6·1 cub. cent., of

which 4·6 were absorbed by potash, and the remainder proved to be combustible. Hence, up to this date, there has been no appreciable evolution of free Nitrogen. In order to see whether the organic matter present would reduce a nitrate, with the evolution of free Nitrogen, about 5 grammes of saltpetre were now put into the vessel, and it was replaced in the same room as before.

May 3, 1859.—Several times since December 12, 1858, when the nitrate of potash was put in, the vessel has been warmed up to 30° C. ; but up to this date very little gas has been evolved.

May 25, 1859.—Still very little gas evolved ; 4 cub. cent. only collected, one-fourth of which was carbonic acid, and the remainder was combustible. The vessel was now placed in the sunlight again, but up to the middle of June no further evolution of gas had taken place. The fluid still contained nitrate of potash. The vessel was then half filled with oxygen in order to see if this would cause a renewal of the decomposition. After ten days a portion of the gas was examined, when it was found that not one-fourth of the supplied oxygen had been consumed—a result which was quite unexpected. The total gas being removed, the vessel was again nearly filled with oxygen, driving out the greater part of the fluid, and leaving the partly decomposed seeds in an atmosphere of this gas. The apparatus so arranged was placed in the sunlight, and remained there during some very warm weather.

July 12, 1859.—The gas collected contained in 100 parts—

Carbonic acid.	Oxygen.	Nitrogen.
20	79	1

By accident a small quantity of air was admitted into the vessel, so that the analysis can only be taken to show how exceedingly slow was the oxidation of organic matter which had been treated as this had been.

On the removal of the matters from the vessel, the Beans were found to possess much of their original firmness and solidity. The other seeds, though they retained their form, were softer, and they had evidently undergone a more complete decomposition. They emitted very little odour, which was not unpleasant.

There can be no question as to the absence of any evolution of free Nitrogen during the long period that these three descriptions of seed were under experiment. A very small proportion of the combined Nitrogen present would, if set free, have been sufficient to fill the vessel with gas. But, as has been seen, only a few bubbles of gas were evolved during several months.

Several other experiments were made upon the products of the decomposition of organic matter, in the first stages of the process. In Table X., which follows, are given the amounts, and the composition, of the gas obtained from decomposing organic matter in a few out of a number of cases in which we have had occasion to observe them—including, for comparison, some of the results already referred to. The decomposition took place in water, in vessels similar to that used in the experiments last described (fig. 7, Plate XII.).

TABLE X.—Showing the products of the action of the reducing forces exercised in the decomposition of Nitrogenous organic matter, as exhibited by the composition of the gases evolved.

Description of Organic matter subjected to Decomposition.	Total Gas collected.	Composition of the Gas, Per Cent.		
		Carbonic acid	Hydrogen.	Nitrogen.
	cub. cent.			
a. Wheat, Barley, and Bean seed ...	400	64·87	34·83	0·30
		64·54	35·46	traces.
b. Turnip plant; root with leaves...	166·2	76·23	22·91	0·87
c. Turnip plant; root with leaves...	162·2	68·83	23·93	7·24
d. Turnip plant; root with leaves...	123·6	68·06	25·63	6·31
		67·52	25·43	7·05
e. Turnip plant; root with leaves...	41·2	64·95	14·66	20·39

The first experiment (*a*) is that which has been considered above. In all the other cases about two ounces of young Turnip plant, the root and leaves together, were operated upon. They were exposed in similar vessels to those used in the other experiments, from August 29 to October 5. At the termination of this period the structure of the plant was almost entirely destroyed; and there remained only a mass of decomposed matter deposited at the bottom of the vessels. The evolution of gas had entirely ceased.

The Turnip plant (*b*) was exhausted of its gas before exposure; and, as will be seen, there was, under these circumstances, a very small quantity of free Nitrogen found at the termination of the experiment.

All the other Turnip plants were submitted to decomposition without previous exhaustion; and hence the amount of Nitrogen eventually found. In the last experiment (*e*) there is a much larger percentage of Nitrogen than in the other cases. But the total quantity of gas was much less; and the comparison of this result with the others shows that there was an almost constant actual quantity of Nitrogen in the several cases, doubtless due to that existing within the plant at the commencement of the experiment. Hence it appears that, in the absence of free oxygen, no free Nitrogen is evolved from the nitrogenous compounds of the plant.

At all events the entire cessation of the evolution of gas after the decomposition has gone on for a few days, shows that the presence of free oxygen is essential to the evolution of Nitrogen, as it is conducive to that of carbonic acid. The loss of Nitrogen indicated in Tables VII. and IX. must be considered, therefore, to be the result of an oxidizing process.

We shall have to allude again to the results given in Table X. when we come to discuss the question of the formation of ammonia from the free Nitrogen of the air, and the nascent hydrogen evolved during the decomposition of organic matter.

In order to examine the character of the decomposition of organic matter in oxygen gas, an investigation was undertaken, which, owing to the difficulty of getting the requi-

site apparatus sufficiently air-tight, was not followed up to the extent which had been intended.

The plan proposed was, to place the organic matter in an atmosphere of pure oxygen, and to afford a constant supply of the gas as it became converted into carbonic acid, and was absorbed by a solution of caustic potash.

The results obtained go to show that, in the presence of free oxygen, Nitrogen gas is evolved. But as the investigation is as yet so incomplete, owing to the circumstance above alluded to, we prefer not to give the results until we can confirm them by a more extended series of experiments under more favourable conditions.

Taking together the results of all the experiments which have been made upon the decomposition of nitrogenous organic matters, they obviously point to a serious difficulty in the way of experiments made upon the question of the assimilation of free Nitrogen by plants. It is not possible to conduct any such experiments without exposing nitrogenous organic matter to conditions more or less analogous to those under which the loss of Nitrogen recorded in Tables VII. and IX. took place. For although, as Boussingault has shown, there may be no loss of Nitrogen during germination, yet during the entire period of the growth of a plant, certain portions of the vegetable substance may be subjected to conditions favourable to the decomposition of its nitrogenous compounds, and to the evolution of free Nitrogen.

As illustrative of how far these conditions are likely to be operative in the manner indicated, the following results, made with Wheat, Barley, and Oats respectively, are very instructive. Seeds of the three plants were sown, each in precisely the same kind and amount of soil, &c., as employed in the experiments on the assimilation question. The three pots were placed beneath a large glass shade, 16 inches in diameter, which fitted into the groove of a stone-ware lute-vessel, into which sulphuric acid was poured to exclude the access of external air. The whole stood on a table in the diffused light of the laboratory. The plants were at first supplied with distilled water; but with no carbonic acid beyond that which might be contained in the water. These conditions afforded all that was necessary for germination and growth, with little opportunity for the assimilation of free Nitrogen, even were this possible in the more favourable conditions of sunlight. Yet the conditions were more than ordinarily favourable to the decomposition of nitrogenous compounds, provided this would take place, under certain circumstances, during the growth of the plant. The succulent character of the stems and leaves so grown in the shade, would render the nitrogenous matters more liable to decomposition than in the case of the more firm and hardened stems of plants grown in sunlight.

Eight seeds of each plant were sown; and in a few days all came up, and grew very rapidly in height, without much tendency to development and expansion of leaf. The plants were all very much alike—tall, slender, delicate, and having the peculiar pale-green colour common to plants deprived of sufficient sunlight. In several other expe-

riments it was found that plants which had proceeded for some time in this delicate form of growth, immediately ceased this predominant upward tendency when removed into sunlight: then, after remaining stationary for a few days, during which time the extremities of the long delicate leaves lost their vitality, the plants commenced a new order of growth, producing many more leaves, which were much shorter and broader than the earlier ones; the stems also became thicker and more dense than before.

The seeds were put in on May 17 (1858); and on June 10 following, the plants had ceased to grow. Several of the long slender stems were too delicate to support themselves, and began to fall over. All the plants presented much the same appearance, each with a small sheath without any leaf at the base, and three leaves higher up—the two lateral ones being very long, from 8 to 12 inches, and the terminal ones, not unrolled, from 3 to 4 inches long from the axial of the next leaf below, the whole plant being from 7 to 11 or 12 inches high. On removing them from the soil, it was found that the roots were distributed very little through it. They consisted of short fibrils, with divaricated branchlets, extending principally around the seeds, and seldom more than 2 or 3 inches through the soil. The plants were so very much alike, that it was difficult to distinguish the different kinds. Fig. 9, Plate XII., is reduced from a sketch of one of these attenuated plants.

The following Table gives the quantitative particulars of the experiments.

TABLE XI.—Showing the effect of Germination, and Growth without direct Sunlight, or extraneous supply of Carbonic Acid or combined Nitrogen, upon the combined Nitrogen originally provided in the Seed.

Duration of experiment twenty-four days—from May 17 to June 10, 1858.

Particulars of the seed sown.					Dry vegetable matter produced.			Nitrogen.			
Description.	No.	Weight, fresh.	Weight, dry.	Nitrogen.	Stems.	Roots.	Total.	In stems and roots.	In soil and pot.	In total products.	Gain or loss.
		gramme.	gramme.	gramme.	gramme.	gramme.	gramme.	gramme.	gramme.	gramme.	gramme.
Wheat	8	0·4865	0·4077	0·00790	0·320	0·140	0·460	0·00697	0·0012	0·00817	+0·00027
Barley	8	0·3875	0·3234	0·00573	0·290	0·160	0·450	0·00570	traces	0·00570	−0·00003
Oats	8	0·3475	0·2900	0·00640	0·355	0·060	0·415	0·00640	traces	0·00640	+ traces

The weights given for the roots are a little too high, owing to their not having been washed entirely free from soil, the principal object being to ensure a correct result with regard to the Nitrogen which long washing might have endangered, or at least rendered less easy. There is, however, evidently a slight gain of dry matter, which, so far as its carbon is concerned, was doubtless due to carbonic acid in the distilled water, of which about 500 cub. cent. were added to each pot at the commencement of the experiment. None was added during the progress of the experiment; but the soil was moist when the plants were taken up.

The rapid growth of the plants, the short period of their contact with the soil, the

very limited distribution of the roots, and the fact that no water was added during growth, which would tend to distribute any soluble or otherwise easily transportable matters, are conditions all consistent with the almost total absence of Nitrogen in the soil.

Lastly, in regard to the results in the Table (XI.), the final column, showing the gain or loss of Nitrogen, affords us the means of judging how far the molecular actions by which free Nitrogen was given off in the cases of the experiments upon the decomposition of nitrogenous organic matter are likely to interfere with the results of our investigations on the question of assimilation of free Nitrogen by plants. It is seen that, in the experiments now under consideration, no free Nitrogen was given off during the process of germination and growth. At least, the assumption that free Nitrogen was given off implies the still more improbable one, that, under the circumstances detailed, assimilation of free Nitrogen has taken place ; whilst the adoption of these two assumptions necessitates the yet more improbable one, that these two independent actions bear a most definite relation to each other—in fact, that the amount of free Nitrogen assimilated is exactly equal to that given off during decomposition.

It would appear, therefore, that we may rest satisfied that our results in regard to the question of assimilation will not be affected by a loss of free Nitrogen as the result of the decomposition of nitrogenous organic matter, so long as that matter is subjected to the ordinary process of germination, and exhaustion to supply materials for growth. Our results in regard to the products of decomposition of nitrogenous organic matter do, indeed, point to the danger of using nitrogenous organic manure in such experiments, and to the error that might occur from seeds decomposing in the soil instead of growing, or from the decomposition of dead leaves of old roots, or of nitrogenous organic excretions ; but they do not afford any evidence of what takes place within the range of the action of the living plant. And, judging from the amount of free Nitrogen evolved when, as in the experiments on decomposition, so large a proportion of the nitrogenous organic matter was decomposed, we may form some idea of the probable extent of such evolution when, as in experiments where vegetable growth is involved, and where the only nitrogenous organic matter supplied is that in the seed sown, but a small proportion of the total nitrogenous matter undergoes decomposition.

In relation to this question, it should be borne in mind that, in the cases where the large evolution of free Nitrogen took place, the organic substances were subjected to decomposition for a period of about six months, during which time they lost three-fourths of their carbon. In the experiments on the question of the assimilation of free Nitrogen, however, but a very small proportion of the total organic matter is subjected to decomposing actions apart from those associated with growth, and this for a comparatively short period of time, at the termination of which the organic form is retained, and therefore but little carbon is lost. It would appear, then, that we need not fear any serious error in our experiments in regard to the latter question, arising from the evolution of free Nitrogen in the decomposition of the nitrogenous organic matters

involved. On the other hand, the facts adduced afford a probable explanation of any small loss of Nitrogen which may occur when seeds have not grown, or when leaves, or other dead matter, have suffered partial decomposition.

F.—*The mutual relations of Gaseous Nitrogen and the Nascent Hydrogen evolved during the decomposition of organic matter.*

The importance attached by MULDER, and others after him, to the action of nascent hydrogen, evolved in the decomposition of organic matter, upon gaseous Nitrogen, as a source of ammonia, is such as to require that we should refer to the subject here, in the course of the discussion of the conditions possibly affecting the supply of combined Nitrogen to our experimental plants. The results given in the last sub-section (pp. 509–511), leave no doubt of the evolution of hydrogen during the decomposition of organic matter. They suggest, therefore, the possibility that such an evolution may take place in any decomposition of organic matter involved in our experiments on the assimilation of free Nitrogen by plants, and hence prove a source of ammonia to them.

That nascent hydrogen may, under certain circumstances, combine with gaseous Nitrogen, has long been admitted. But the view so prominently put forth by MULDER * and some others, that those circumstances occur in the evolution of nascent hydrogen accompanying the decomposition of organic matter, requires confirmation.

If only a very small part of the hydrogen evolved in the decomposition of organic matter were to form ammonia with the Nitrogen gas which must always be in most intimate contact with it, the amount of ammonia formed in this way would be enormous. Peat bogs, cesspools, and all stagnant water pregnant with organic matter, as well as many soils, would be constantly so accumulating ammonia. The extensive forests in different parts of the world, which have been annually depositing a coating of leaves upon the surface of the soil for thousands of years, must also have been a very fertile source of ammonia, as the leaves have gradually decayed under the influence of moisture and confined air beneath the succeeding layers. And when we contemplate the amount of decomposition that must have corresponded to the very exuberant growth of former geological periods, as manifested in the remains exhibited in our coal beds and limestones, we see a source of ammonia, if formed in the manner now under consideration, which would be incalculable.

The results given in the last subsection (E), upon the decomposition of nitrogenous organic matter, favour the view that the hydrogen evolved in such decomposition does not form ammonia with the Nitrogen of the air. The assumption that it did so, implies that the nascent hydrogen was capable of uniting with free gaseous Nitrogen (forming ammonia) under circumstances in which its affinities were not sufficiently powerful to prevent Nitrogen compounds very similar to ammonias (and which are easily transformed into them) from giving up Nitrogen in the free state. It implies also, that the nascent hydrogen can act upon ordinary Nitrogen, when it cannot do so upon this nascent Nitro-

* Chemistry of Vegetable and Animal Physiology, pp. 111–114, 149–152, &c.

gen of the decomposing nitrogenous body. Or, if it did act upon the latter in preference to the former, there would either be no free Nitrogen finally evolved, or, in case of Nitrogen being lost in the free state, it would be obvious that there had been less nascent Nitrogen converted into ammonia than had been liberated from its combinations, and hence that, as a resultant, there would be a *loss* and *not a gain* of combined Nitrogen due to the decomposition.

The fact that, in our experiments upon the gas evolved by vegetable matters in a state of decomposition, both free Nitrogen and free hydrogen were given off, bears strongly upon this question. The Nitrogen evolved has been in most intimate contact with the hydrogen given off. It has, indeed, been in the identical cells by the decomposition of the walls or contents of which the hydrogen was set free ; yet both appear as gas.

From the above considerations it would appear that we need be under little apprehension of error in the results of our experiments on the question of the assimilation of free Nitrogen by plants, arising from an unaccounted supply of ammonia formed under the influence of nascent hydrogen, given off in any decomposition of the organic matter involved in the experiment.

Summary Statement of the Results of the foregoing consideration of the conditions required, or involved, in Experiments on the question of the assimilation of free Nitrogen by Plants.

Before entering upon the discussion of the results of our direct experiments upon the question whether or not plants assimilate free Nitrogen, it will be well, for the sake of perspicuity, to give a very brief enumeration of the results arrived at in the foregoing Sections I. and II. (Part II.), relating to the conditions of experiment required, and to the collateral investigations involved, in the inquiry. They may be stated as follow :—

1. Conditions of soil or matrix which are both adapted for healthy growth and are consistent with the other requirements of the investigation can be attained (Section I. Sub-sections A, p. 470, and L, p. 484).

2. The requirements of the experiment in regard to the selection of seeds or plants for growth, to the nutriment to be supplied in the soil, to the water, to the atmosphere, to the carbonic acid, and to other conditions involved, can be satisfactorily met (Section I. Sub-sections B–J, inclusive, pp. 472–481, and L, p. 484).

3. The conditions of experiment adopted have several advantages over some of those which have been suggested, or adopted, by others (Section I. Sub-section K, pp. 481–483).

4. The mutual actions of the soil, air, organic matter in the soil or in the plant, are not such as to be likely to affect the result of the experiment, by yielding to the plants a quantity of combined Nitrogen not taken into account. The influence of Ozone as a possible element in these actions would be less, in the circumstances of the experiments

on assimilation, than in those of experiments the results of which showed no appreciable formation of compounds of Nitrogen (Section II. Sub-sections A–C, pp. 484–497).

5. The fact of the evolution of free Nitrogen during the decomposition of nitrogenous organic matter has been confirmed by experiment ; but the circumstances of the decomposition in which the evolution of free Nitrogen was observed, when compared with those involved in an experiment on the question of assimilation, are not such as to lead to the conclusion that there would be a loss of Nitrogen from this source in experiments of the latter kind, unless in certain exceptional cases, in which it might be presupposed (Section II. Sub-section D, pp. 497–508).

6. The forces, by virtue of which free Nitrogen is eliminated from its compounds in organic matter, are of an oxidizing character ; they are not exercised in the absence of oxygen. They are not likely to be operative in connexion with growing vegetable matter (Section II. Sub-section E, pp. 509–515).

7. Although it is known that, under certain circumstances, nascent hydrogen may combine with free Nitrogen and form ammonia, it is questionable whether the nascent hydrogen eliminated during the decomposition of vegetable matter will be in the conditions to effect such a combination ; nor are the circumstances of our experiments on the question of the assimilation of free Nitrogen by plants such as to lead to the supposition, that an error in the results can arise from the formation of any ammonia under the influence of the action supposed (Section II. Sub-section F, pp. 515, 516).

Section III.—CONDITIONS OF GROWTH UNDER WHICH ASSIMILATION OF FREE NITROGEN BY PLANTS IS MOST LIKELY TO TAKE PLACE ; DIRECT EXPERIMENTS UPON THE QUESTION UNDER VARIOUS CIRCUMSTANCES OF GROWTH.

A.—*General consideration of conditions of growth.*

We have thus far discussed, in some detail, the arrangement adopted in our experiments on the question of the assimilation of free Nitrogen by plants, and the collateral points involved in the relation of gaseous Nitrogen to vegetation. In regard to the latter, we have dwelt particularly on those which relate to the sources of available Nitrogen to plants, and which, therefore, may tend to influence the quantitative results which we may obtain by the methods of experimenting followed. It remains to consider what are the circumstances under which it is most probable that free Nitrogen may be assimilated, provided the assimilation can take place at all.

The demonstration of the fact, that the process of cell-development could go on in the presence of free Nitrogen without the latter becoming incorporated into the cell wall, or into the contents of the cell, as a nitrogenous compound, would not carry with it the demonstration that free Nitrogen could, under no conditions of growth, undergo such change. Our aim should be, therefore, to seek the most probable circumstances

for such change; and if we find that in them free Nitrogen is assimilated, we should then trace up the question through the circumstances in which such assimilation is less likely to take place.

If, on the contrary, we find that free Nitrogen is not assimilated under the circumstances which appear the most favourable for such an action, we may either generalize for other conditions from the negative results so obtained, or we may extend our experiments in order to widen the basis of our generalizations.

In the consideration of what are the cases in which the assimilation of free Nitrogen is most likely to take place, two important classes of conditions present themselves :—

1. Those which relate to the supply of combined Nitrogen at the disposal of the plant.

2. Those which relate to the activity of growth and stage of development of the plant.

These two questions, though logically distinct, are physiologically blended; for it may happen that a certain activity of growth, or certain stages of development, can only be attained by a given supply of combined Nitrogen beyond that contained in the seed.

If we examine these conditions a little more closely, we see that they give us the following possible cases for the assimilation of free Nitrogen by the plant :—

1. The plant may be able, in the process of cell-formation, to derive the whole of its Nitrogen from that presented to it in the free state.

2. It may be capable of assimilating a part of its Nitrogen from that presented to it in the free state, provided it be supplied with only a part of its required amount in some form of combination.

3. It may assimilate free Nitrogen in the presence of an excess of combined Nitrogen.

Again :—

1. It may be capable of assimilating free Nitrogen in the earlier stages of its development.

2. It may be so at the most active period of its growth.

3. It may when near the period of its maturity.

Combinations of these several circumstances present at least nine special cases, in one of which, if at all, an assimilation of free Nitrogen might take place without its doing so in any of the others. The question arises, how are we so to arrange our experiments as to include the greatest number of these cases, and those in which the assimilation of free Nitrogen is the most likely to occur ?

The obviously most probable circumstances for the assimilation of free Nitrogen at any stage of development of the plant, are those in which it is brought to that stage in a healthy condition, and then deprived of all sources of combined Nitrogen. It is hardly to be supposed that an assimilation of free Nitrogen would take place if there were an excess of combined Nitrogen at the disposal of the plant; for, if we suppose that the molecular and vital forces are at the same time acting upon Nitrogen supplied by these two sources, in a manner tending to force that from both into the constitution

of the living organism, it is only consistent with our established notions of force, that the form which yields with the greatest ease will yield first, and that, if its supplies be in sufficient quantity, it only will yield in an appreciable degree to the force applied.

If, on the other hand, the forces involved in vegetable growth, tending to form nitrogenous compounds, are capable of appropriating free Nitrogen only in the presence of a certain amount of assimilable combined Nitrogen, then the question of deciding upon the proper proportion of combined Nitrogen to effect the assimilation of that provided in the free state would seem, *à priori*, to present serious difficulty. For if the plant cannot assimilate free Nitrogen either in the presence of an excess of combined Nitrogen, or without the aid of a certain amount of it, it would, at first sight, appear that there might be some difficulty in so arranging an experiment as to hit the proper medium.

But within a certain range of conditions this supposed difficulty would not occur. If the assimilation of free Nitrogen be possible only as the result of the assimilating forces acting upon it in the presence, or with the aid, of a certain amount of combined Nitrogen, then, when the quantity of combined Nitrogen has become too small, the point must have been passed at which the maximum amount of free Nitrogen would be assimilated in relation to the then existing supply of combined Nitrogen. Hence, the analysis of a plant at the period at which its growth ceased in consequence of the falling short of the relative supply of Nitrogen in the combined form, would show whether or not an assimilation of free Nitrogen had taken place as the result of either of the conditions referred to in the last paragraph.

If, however, the plant cannot assimilate free Nitrogen under the conditions of the supply of combined Nitrogen just referred to, unless it has attained a certain vigour of growth, or reached a certain stage of its development, and the supply of combined Nitrogen has been insufficient to bring it to the supposed requisite point, then no assimilation of Nitrogen would take place, even though it might do so provided the proper stage of growth had been passed. To the cases here supposed we shall recur further on.

If the assimilation of free Nitrogen can take place at all periods of the growth of the plant, and in the absence of all sources of combined Nitrogen, the solution of our question becomes much more simple than in either of the cases above referred to.

In illustration of the fact that, within a certain range of other conditions, there can be no difficulty in securing in an experiment those involved in the presence of an excess, of a certain limited quantity, or of no combined Nitrogen, attention may be directed to the phenomena of vegetable growth when seeds are grown in a soil and atmosphere free from combined Nitrogen.

Under the circumstances supposed, all the conditions with regard simply to the relative quantity of combined Nitrogen are afforded. Thus, when the seed is first sown, it contains within itself an excess of combined Nitrogen, so far as the demands of the plant at the time are concerned. The rapidity with which the Nitrogen of the seed can

be used, in the growing process, is seen in the results of the experiment in regard to the question of the decomposition of Nitrogenous matter during growth, as given in Table XI. (p. 513); and the extent to which it can carry the growth of the plant is illustrated in that experiment, as well as in others, to which we shall presently refer, relating to the question of assimilation itself. It is obvious that, during a part of the time at the end of which the plant has reached the limit of its supply of combined Nitrogen, it has had at its disposal an excess of combined Nitrogen for its immediate wants. It has then passed through a stage in which the particular relation of combined to free Nitrogen implied in another of our assumed conditions must have existed. It must finally have reached a point at which only free Nitrogen was presented to it.

If an analysis of the plant at the termination of the last-mentioned period showed no increase of Nitrogen, the result would afford conclusive evidence against the possibility of the assimilation of free Nitrogen under a wide range of conditions. If, on the contrary, a gain of Nitrogen were indicated, the question would still be open, to which of the several conditions to which the plant had been subjected it owed the increase found. But this question we need not discuss until we have recorded the results of our experiments on the point.

B.—*Direct experiments on the question of the assimilation of free Nitrogen by plants.*

We have thus far discussed the methods of experimenting to be adopted, the results of certain collateral inquiries, and the several conditions under which the assimilation of free Nitrogen by plants may be the more or the less likely to take place. We have thus endeavoured to eliminate all known sources of error, and to acquire the means of forming an estimate of the possible influence of certain unknown quantities, and so, as far as practicable, to reduce the solution of our question to that of a single point to be tested by direct experiment. It remains to consider the experimental evidence relating to this last and final point.

An investigation requiring several hundred analyses, and a series of observations made at intervals of a few days, through periods of several months, involves an amount of recorded detail much too voluminous for full publication. An abstract of the most important portions of the records will, however, be given for reference in the Appendix. A statement of the methods of analysis adopted, with illustrations of the limits of accuracy reached, together with a condensed summary of the details of growth of the plants, will there be given.

In the selection of the plants to submit to our adopted conditions of experiment, we have been guided by several considerations :—

1. To have such as would be adapted to the conditions of temperature, moisture, &c., to which they were to be subjected.

2. To have such as were of importance in an agricultural point of view.

3. To acquire the means of studying any difference, in reference to the point in question, between plants which belong respectively to the two great Natural Orders,

the Graminaceæ and the Leguminosæ, which in some points of view, appear to differ so widely in their demands upon combined Nitrogen provided within the soil.

4. To take such as had already been experimented upon, with such conflicting results, by M. Boussingault and M. G. Ville.

We shall first consider the results obtained with plants grown without any other supply of combined Nitrogen than that contained in the seed sown.

I.—*Experiments in which the plants had no other supply of combined Nitrogen than that contained in the seed sown.*

The following Table (XII.) gives, at one view, a summary of the numerical results obtained under this head; see also figs. 1–6, Plate XV., which are reduced from careful drawings taken of six out of the nine Graminaceæ experimented upon, and illustrate the character and extent of growth attained under the conditions in question.

After the full discussion in the foregoing pages of the circumstances under which the results recorded in the Table just given were obtained, but little need be said in pointing out their bearings upon the question at issue. The column showing the gain or loss in each experiment speaks for itself. In judging of the results of the experiments of 1857, the remarks made in discussing the results of Table XIV. (p. 532), with regard to the slates used as lute-vessels in that year, must be taken into consideration. The source of error referred to being obviated in the experiments of 1858, the results of 1857 acquire a greater value, as confirming those of the latter year, than, standing alone, they would possess.

The difference between the results obtained with soil and with pumice as matrix, in 1857, are not such as to lead us to attach any importance to them, or to attribute them in any way to the difference of matrix in question. The two experiments may therefore simply be considered as duplicates. Indeed, the character of the results in the one experiment with Wheat, and in the two with Barley, in 1857, was so similar, that the three experiments may be considered as triplicates.

Graminaceous Plants.

It will be observed that the largest gain of Nitrogen in the three experiments with Graminaceæ in 1857 was 0·0026 gramme. Keeping in view the probable source of error due to the use of slates in that year, and the difference of result in 1858 when slates were not employed, and, again, considering the fact that so small an amount of Nitrogen had to be determined in such a large amount of soil (0·003 gramme or less of Nitrogen in about 1500 grammes of soil), it seems indeed more than questionable whether the gain should not be attributed to the errors of experiment or analysis alluded to. In fact, we can but conclude that, under the circumstances of growth of the Graminaceous plants to which Table XII. relates, there has been no assimilation of free Nitrogen.

It should also be noticed that, even when a gain of Nitrogen in the total products is observed, there is, in no case, more Nitrogen in the plant itself than in the original

TABLE XII.—Showing the Numerical Results of Experiments to determine whether Plants supplied with no other combined Nitrogen than that contained in the original seed assimilate Free or uncombined Nitrogen.

General Particulars of the Experiments.				Numerical particulars of the seeds sown.					Numerical results at the termination of the Experiments.					Summary.		
				Number.		Weights.			Dry weights of Plants produced.	Nitrogen.				Nitrogen in seeds sown.	Nitrogen in total products.	Gain or loss of combined Nitrogen.
Year, &c.	Description of Plant.†	Description of soil or Matrix.	Period of Experiment.	Sown.	That grew.	Fresh.	Dry.	Nitrogen.		In total Plant.	In Soil.	In Pot.	In condensed water.			
Graminaceæ.																
						gramme	gramme.	gramme	gramme	gramme	gramme	gramme	gramme	gramme.	gramme.	gramm
1857...	Wheat (1) ...	Prepared soil ...	May 16—Oct. 3	6	5	0·3658	0·3065‡	0·0080	1·412	0·0072	traces	traces	.....	0·0080	0·0072	−·0008
	Barley (2) ...	Prepared soil ...	May 20—Aug. 24	6	6	0·3230	0·2698	0·0056	0·810	0·0047	0·0024	0·0001	.....	0·0056	0·0072	+·0016
	Barley (3) ...	Prepared pumice	May 20—Aug. 25	6	6	0·3233	0·2698	0·0056	0·925	0·0045	0·0030	0·0007	.....	0·0056	0·0082	+·0026
1858...	Wheat (1) ...	Prepared soil ...	April 27—Oct. 25	8	8	0·4815	0·4035	0·0078	1·740	0·0056	0·0025		traces	0·0078	0·0081	+·0003
	Barley (2) ...	Prepared soil ...	April 27—Aug 18	8	6	0·3860	0·3221	0·0057	0·560	0·0031	0·0027		traces	0·0057	0·0058	+·0001
	Oats (3) ...	Prepared soil ...	April 27—July 13	8	8	0·3425	0·2858	0·0063	1·148	0·0042	0·0011		0·0003	0·0063	0·0056	−·0007
1858, A.*	Wheat	Prepared soil ...	June 11—Nov. 6	8	7	0·4810	0·4031	0·0078	1·060	0·0041	0·0033		0·0004	0·0078	0·0078	0·000
	Barley	Prepared soil ...	June 11—Nov. 6	8	8	0·3840	0·3240	0·0057	0·710							
	Oats	Prepared soil ...	June 11—Nov. 6	8	7	0·3450	0·2879	0·0064	0·690	0·0038	0·0021		0·0004	0·0064	0·0063	−·0001
Leguminosæ.																
1857.....	Bean (4)	Prepared soil ...	May 16—July 5	2	2	1·8907	1·4984	0·0796	7·028	0·0629	0·0146	0·0016	.....	0·0796	0·0791	−·0005
1858...	Bean (5)	Prepared soil ...	June 11—Aug. 23	3	3	1·8700	1·4820	0·0750	4·875	0·0735	0·0016		0·0006	0·0750	0·0757	+·0007
	Pea (6)	Prepared soil ..	June 5—Aug. 24	3	3	0·6436	0·5405	0·0188	0·970	0·0102	0·0056		0·0009	0·0188	0·0167	−·0021
Other Plants.																
1858.....	Buckwheat (7)	Prepared soil ...	Aug. 20—Oct. 28	24	13	1·0000	.....	0·0200	0·450	0·0070	0·0108		0·0004	0·0200	0·0182	−·0018

* These experiments were conducted in the apparatus of M. G. VILLE.

† The numbers given in brackets are those under which the respective plants are described in the "Abstract of the Records of growth of the Plants," given in the Appendix, p. 548 *et seq.*

‡ The percentage of dry matter in the seed was not determined in this case; it is therefore assumed to be the same as in the wheat used in 1858, from which it would certainly not differ at all materially.

seed,—the gain appearing only when the Nitrogen in the soil and pot is taken into account. It will be remembered that the results of the experiments on the question whether there was an evolution of Nitrogen during germination and growth (Table XI., p. 513) showed how completely the plants could appropriate the Nitrogen of the seed from which they grew, leaving only traces of it in the soil. Again, the experiments on the decomposition of Nitrogenous organic matter (Tables VIII. and IX., p. 506) have shown how thorough was the decomposition coincident with the passage of any large percentage of the combined Nitrogen of the substance into the soluble state of ammonia. Taking together these facts, we have strong grounds for assuming that at least a part of the Nitrogen found in the soil, in the cases where there was a gain of it in the total products, has never been in actual connexion with the plant at all. Indeed, in view of the facts just referred to, any gain of Nitrogen in connexion with the plant, without there being a larger quantity of Nitrogen in the plant itself than that provided in the seed, would be very questionable evidence upon which to establish the fact of the assimilation of free Nitrogen.

But the results obtained with Graminaceæ in 1858, when all possible sources of error which the experience of the previous year had suggested had been eliminated, point, without exception, to the fact that, under the circumstances of growth to which the plants were subjected, no assimilation of free Nitrogen has taken place. The regular process of cell-formation has gone on ; carbonic acid has been decomposed, and carbon and the elements of water have been transformed into cellulose ; the plants have drawn the nitrogenous compounds from the older cells to perform the mysterious office of the formation of new cells (see Notes on growth, Appendix, pp. 559, 561) ; those parts have been developed which required the smallest amount of Nitrogen ; and all the stages of growth have been passed through to the formation of glumes, pales, and awns for the seed. In fact, the plants have performed all the functions that it is possible for a plant to perform when deprived of a sufficient supply of combined Nitrogen. They have gone on thus increasing their organic constituents with one constant amount of combined Nitrogen, until the percentage of that element in the vegetable matter is far below the ordinary amount of it—that is, until the composition indicates that further development had ceased for want of a supply of available Nitrogen.

Throughout all these phases, water saturated with free Nitrogen has been passing through the plant ; nitrogen dissolved in the fluid of the cells has constantly been in the most intimate contact with the contents of the cells and with the cell-walls. The newly forming cell, stunted in its development for want of assimilable Nitrogen, has nevertheless been surrounded by free Nitrogen. Its delicate membranes have been saturated with water, itself saturated with free Nitrogen ; and such are the laws in accordance with which the absorption of gases, and the transmission of liquids through membranes take place, that the instant a part of the Nitrogen of the saturated fluid became assimilated, the equilibrium would be restored, by the penetration into the cell of other saturated liquid, and the re-saturation of that from which Nitrogen had been with-

drawn. It would hardly be supposed that, under such circumstances, the process of cell-formation could go on without the assimilation of free Nitrogen, provided any forces were exerted in the cell the tendency of which was to fix free Nitrogen in the organism of the plant.

One fact, briefly alluded to above, we wish to call more special attention to, as affording strong evidence of the absence of the power on the part of these cereal plants to appropriate free Nitrogen—namely, the very large development of the root, requiring but little Nitrogen compared with that of other parts. It was observed, in the experiments of 1857, that several of the cereal plants developed a large proportion of root; but the danger of accident in analysis was such, that we hesitated to double the risk of losing the entire result by analysing the root and the portion of the plant above ground separately. They were, therefore, thoroughly mixed, and the mixture was carefully divided; so that, in case of accident, a duplicate was at our disposal, and in case of all going well, confirmatory evidence was obtained. So very marked, however, was the great development of root in the cereals of 1858, that, in several cases, it was analysed separately from the other parts of the plant. The remarkable result was obtained, that this great root-development was carried on (in two, at least, out of the three instances in question) with a consumption of an almost incredibly small amount of Nitrogen, as the figures given in the following Table will show :—

TABLE XIII.

Description of Plant. 1858.	Dry Matter in Produce (at 100° C.), grammes.			Nitrogen in Produce (grammes).			Per cent. of Total Dry Matter in Roots.	Per cent. of Total Nitrogen in Roots.
	In Stems, &c.	In Roots.	In Total Produce.	In Stems, &c.	In Roots.	In Total Produce.		
Wheat (1)	0·890	0·850	1·740	0·0039	0·0017	0·0056	48·85	30·36
Barley (2)	0·400	0·160	0·560	0·0027	0·0004	0·0031	28·57	12·90
Oats (3)	0·798	0·350	1·148	0·0040	0·0002	0·0042	30·49	4·76

The large proportion of root and its small proportion of Nitrogen, as here exhibited, are equally remarkable. Whether this great power of the plant to develop root be due to the fact that the process of cell-formation in the root requires less of the nitrogenous protoplasmic compound, or to the fact that, floating in water as these roots generally were, that fluid facilitated the withdrawal of the nitrogenous constituents resulting from the decomposition of protoplasma from the old cells, to form new protoplasma for the more active cells, is a question which, though foreign to our present subject, is of considerable interest in a physiological point of view. The fact that the roots from the base of the stem penetrated the soil, giving off very few branches into it, but immediately on reaching the water at the bottom of the pot exhibited such a remarkable development (see Notes on taking up the Wheat Plants, Appendix, p. 560), is in favour of the inference that the water afforded the necessary conditions for the character of growth referred to.

But, apart from the physiological points just referred to, as already said, this great development of a part of the plant requiring a minimum amount of Nitrogen affords strong evidence of its inability to assimilate free Nitrogen within the range of development possible when no combined Nitrogen is provided beyond that contained in the original seed. It exhibits the great tenacity of growth of the plant, and shows the activity of the vital force, long after the demands of the organism had begun to require more available Nitrogen than was at its disposal. When it is considered how great was the length of time during which the growing cells were exposed to the conditions in question, there would seem to be a combination of circumstances favourable to the exercise of any force tending to bring free Nitrogen into the constitution of the plant. But no such effect is manifested in the results.

The Graminaceæ referred to in the Table (XII.) under the Title of "1858, A.," and which were grown in the enclosing apparatus of M. G. Ville, as already alluded to, give results quite similar in their bearings on the main question to those of 1857 and 1858 already discussed. Being sown later, however, and their period of growth being shorter, they did not manifest such an extraordinary development of root; nor was there so large an amount of vegetable matter produced. Unfortunately the barley grown in M. Ville's Case without artificial supply of combined Nitrogen, was lost by the giving way of the tube in the combustion for the determination of Nitrogen. In its case, therefore, we can only give the amount of the dry matter of the plants produced. But, comparing this with that of the seed sown, and looking to the proportions of Nitrogen in the produce of barley in the other cases, there is no reason to believe that the result would have formed any exception to that indicated in the other experiments.

In concluding our remarks on the results with the Graminaceæ grown without any further supply of combined Nitrogen than that contained in the seed sown, we would beg to refer the reader to the foregoing consideration of the conditions possibly favourable to the assimilation of free Nitrogen (p. 517 *et seq.*).

It will be remembered that, in experimenting with Graminaceæ, including some of the same description as those experimented upon by ourselves, M. Boussingault and M. G. Ville obtained most unaccountably discordant results. It will be seen that our own results, from nine experiments with such plants, go to confirm those of M. Boussingault. In fact, so far as our labours with these plants bear upon their experiments, they could not have given a more decided result.

For representations of some of the Graminaceæ grown without any supply of combined Nitrogen beyond that contained in the original seed, see figs. 1 to 6, Plate XV.

Leguminous Plants.

It still remains to consider the results of our experiments with Leguminous plants grown under similar conditions to those of the Graminaceous ones above discussed, and to see how far they serve to explain the known characteristics of such plants when grown in practical agriculture, to which attention has been directed in Part First of this Paper.

It will be remembered that, under equal circumstances of soil and season, Leguminous crops yield two, three, or more times as much Nitrogen per acre as Graminaceous ones. Yet, whilst the latter are very characteristically benefited by the use of direct nitrogenous manures, the former, yielding so much more Nitrogen, are not so. Again, the Graminaceous crop, requiring for full produce such direct supply of available Nitrogen within the soil, is very much increased, beyond what it would be if it succeeded a crop of the same description, when it follows a Leguminous crop, in which has been carried off so much Nitrogen.

Experiments such as those now specially under consideration can obviously bear upon a few only of the circumstances with which may be connected the causes of this difference between the Graminaceous and the Leguminous crops. Without, therefore, pretending adequately to discuss this wide subject, we will consider it only so far as our immediate facts appear to bear upon it; they seem to limit us to the consideration of the following cases:—

1. The difference may be due to the decomposition of nitrogenous compounds during the growth of the Graminaceous plants, and to the evolution of free Nitrogen.

2. The Leguminous plants may assimilate the free Nitrogen of the air, and thus, not only allow the resources of the soil to accumulate, but also leave within it an additional quantity, in roots and other vegetable débris, from that which has been assimilated, as above supposed.

3. It may be due to the operation of both these causes.

So far as the facts we have already considered go, the difference in question cannot be explained according to the first of the above suppositions; and others, to which we shall have presently to refer, will be seen to afford confirmatory evidence on the point.

With regard to the second supposed explanation, the results we have now to record of our experiments with Leguminous plants are not of themselves sufficient to settle every point which it involves. Reference to the Appendix will show that, in several cases, we failed to get healthy growth with Leguminous plants. A doubt might hence be raised, as to the value of those experiments in which we were successful under circumstances so nearly identical with those of our failures that it was not easy to account for the difference of result obtained. In those cases, however, in which we have succeeded in getting Leguminous plants to grow pretty healthily for a considerable length of time, the results, so far as they go, confirm those obtained with Graminaceæ, not showing in their case, any more than with the latter, an assimilation of free Nitrogen.

In 1857, we commenced several experiments with beans, but they grew well in only one of the shades. These, however (especially one plant out of the two in the same pot), progressed remarkably well for a period of 10 weeks, during which time the amount of carbon was increased five-fold, more than three-fourths of the total Nitrogen of the seed was appropriated, and the plants probably only ceased to grow when the remainder of the latter became so distributed in the soil as not to be available to them.

A reference to Table XII. will show the numerical results of this experiment with beans in 1857.

The beans and peas of 1858, the particulars of which are also given in Table XII., did not grow so satisfactorily as the beans of 1857, last noticed. Yet the beans of 1858 gave more than three times as much organic matter in the produce as was contained in the seed, and they appropriated even a much larger proportion of the Nitrogen of the seed than did those of 1857. The result with the peas was not so satisfactory, owing to the less healthy character and the more limited amount of their growth.

From the fact that these Leguminous plants did not go through a complete course of growth to the flowering process, it may be objected that hence they did not pass certain stages of growth in which they might possibly assimilate free Nitrogen. We shall refer to this objection again further on. At present we confine attention to the important fact, that active growth has taken place—that the process of cell-formation, with the accompanying one of the decomposition of carbonic acid and the fixation of carbon, has gone forward with a deficient supply of combined Nitrogen, and in the immediate presence of free Nitrogen, and yet none of it has been assimilated. The plants have in fact been subjected to a considerable range of the conditions which were considered, *à priori*, to be favourable to the assimilation of free Nitrogen ; and yet this has not taken place.

It is a fact observed in agriculture, that manures rich in organic matter frequently favour the growth of Leguminous crops. We shall not here discuss the question whether these organic manures, as such, act simply as a source of carbonic acid, or of carbon compounds of a more complicated character. We would, however, call attention to the fact that, in the case of the experiments now under consideration, the vital forces were sufficiently energetic to perform the function of cell-development and multiplication, from carbonic acid as its source of carbon ; yet these forces, capable of effecting this result, have been incapable of effecting the appropriation of free Nitrogen.

Buckwheat.

The evidence afforded by the numerical results in the Table XII. relating to this plant is not of so decisive a character as that with regard to the cereals, or even to the Leguminous plants ; for the quantity of dry matter in the produced plants is less than that in the seed sown, whilst the Nitrogen in the plants is little more than one-third that of the seed. But when we come to compare the results of the experiments with Buckwheat grown with and without the supply of ammonia, it will be found that the physiological evidence of the dependence of vegetable growth upon a constant supply of combined Nitrogen is stronger in the case of these plants than in that of the cereals. The small proportion of the total Nitrogen of the seeds which the buckwheat seemed capable of appropriating might lead to the inference that, ceasing to grow with an abundance of combined Nitrogen apparently at its disposal, it had done so for some other reason than the want of available Nitrogen. But this question was set at rest by the fact that, on the addition of an amount of ammonia very small in its contents of

Nitrogen compared with the seed, to plants at the time in a precisely similar condition to those now under consideration, the increase in the rapidity of growth was most marked.

Most of the buckwheat seed sown came up; but about half of the plants lived for only a few days. The remainder, which survived, went through all the stages of development to flowering; but the entire amount of growth was on a very limited scale.

Reference to the last column of Table XII. will show that, under the conditions of growth above described, the buckwheat, like the plants already discussed, indicated no gain of Nitrogen. In fact there appeared to be a loss in the experiment of nearly 2 milligrammes of Nitrogen; and that the result should be to a small extent in this direction may, perhaps, be accounted for by the fact of some of the plants dying early, in consequence of which there may have been a slight evolution of free Nitrogen due to decomposition.

Bearing of the above results on the question of the evolution of free Nitrogen from the Nitrogenous Constituents of plants during growth.

We have thus far only considered the above results so far as they bear upon the question of the assimilation of free Nitrogen by plants. But from the constancy of the amount of combined Nitrogen maintained in relation to that supplied, throughout the experiments, they afford evidence of an important kind in regard to the converse question of whether plants give off free Nitrogen during growth. With no less force than they point to the absence of any assimilation of free Nitrogen, do these results show that, under the circumstances of growth involved, there has been no evolution of free Nitrogen from the nitrogenous compounds of the growing plant. At all events, the assumption that an evolution of free Nitrogen has taken place implies, as in the case of the experiments discussed at pp. 513, 514, the still more improbable one, that there has been an exactly compensating amount assimilated. But since the conditions of the experiments now under consideration were arranged with special reference to the question of assimilation, they necessarily do not embrace all the circumstances which, *à priori*, would be considered the most favourable for the evolution of free Nitrogen during growth.

Various experimenters, from the time of De Saussure until quite recently, have entertained the idea of the probability of the decomposition of nitrogenous compounds, and the concomitant evolution of free Nitrogen, during the growth of plants. We are ourselves engaged in following up the subject, by methods better qualified to settle the question than those adopted in regard to the question of assimilation of Nitrogen. We shall therefore not treat of this subject any further here, than to call attention to the incidental bearing upon it of the results now under consideration.

The fact that there has been no decomposition of nitrogenous compounds and loss of Nitrogen as the result of growth, in the particular conditions to which these experimental plants were subjected, affords little evidence that no such decomposition could take

place under any other circumstances. When supplied with an insufficient quantity of nitrogenous matter, the vegetable organism might not decompose any of that matter; and yet, when an excess of combined Nitrogen was supplied, the decomposition might occur. The results we have given, therefore, afford evidence against the fact of such decomposition only within a very limited range of circumstances of growth. In discussing the results of the experiments the consideration of which we are now about to enter upon, we shall refer to this question again, in connexion with circumstances of growth which we should suppose would be more favourable to an evolution of free Nitrogen by the plant.

II.—*Experiments in which the plants had a known supply of combined Nitrogen beyond that contained in the Original seed.*

We have thus far considered the subject of the assimilation of free Nitrogen, by reference to the results of experiments upon plants grown without any supply of combined Nitrogen beyond that contained in the seed sown. We have found that, under these conditions, we have only been able to study the results of growth of a very limited character. The wheat, and barley, and oat plants, grown in 1858, did indeed progress so far as to produce glumes and pales for seed; but they did not afford the opportunity of studying the results of growth during the period of the formation and the ripening of seeds themselves.

It yet remains to consider, therefore, what may take place under circumstances of a more active and vigorous growth, and at a later stage of development of the plant. When considering the conditions apparently the most favourable for the assimilation of free Nitrogen by plants (p. 517 *et seq.*), we suggested the improbability of such an assimilation taking place in the presence of an abundant supply of combined Nitrogen. If the force of our remarks on this point be admitted, and it be still supposed that an assimilation of free Nitrogen is possible with vigorous growth, only attainable by means of a liberal supply of combined Nitrogen, we seem to be led to the following paradoxical conclusions:—

1. Healthy, active, and vigorous growth are favourable conditions for the assimilation of free Nitrogen by plants.

2. Healthy, active, and vigorous growth can only be attained by keeping within the reach of the plant an excess of combined Nitrogen.

3. Assimilation of free Nitrogen cannot take place in the presence of an excess of combined Nitrogen.

A priori conclusions with regard to the effect of molecular forces, and particularly of those which give rise to vital phenomena, are, however, very unsafe; and we have not been satisfied to rely upon such evidence only, in reference to the question under investigation, as could be afforded by experimenting with plants grown without an extraneous supply of combined Nitrogen. We have found that active and vigorous growth cannot be attained under the conditions provided, when no more combined Nitrogen than that

contained in the seed sown is supplied. We have made a series of experiments, in which such growth was attained by means of a supply of combined Nitrogen beyond that contained in the seed. It remains to see whether, under these conditions of growth, the assimilation of free Nitrogen can take place, and thus the above paradox be obviated by the proof that the last of the three suppositions is incorrect.

It is true that we have pointed out the improbability of an assimilation of free Nitrogen in the presence of an excess of combined Nitrogen only so far as the vital process of the vegetable cell is concerned. In that intermediate process by which oxygen is taken up and carbonic acid formed in the cell, the results due to an excess of combined Nitrogen might be different.

Thus, the more active the growth, the greater must be the amount of newly-formed carbon-matter capable of consuming oxygen, when the plant is removed from the influence of sunlight into the dark. That is to say, the more vigorous the growth in the sunlight, the greater might be the reducing power of the plant in the dark. The greater the reducing power of the plant, the more nearly will the tendency of its molecular forces approximate to an evolution of hydrogen which, in the presence of free Nitrogen dissolved in the fluids of the cell, may tend to form ammoniacal compounds, to be, on the return of light, appropriated by the plant in the exercise of its growing functions. In connexion with this point, it may be here mentioned that in our investi gation of the gases given off by plants under different circumstances, we have had an evolution of oxygen one day as a coincident of growth, and an evolution of hydrogen the next as the result of decomposition.

Our experiments in which the plants have been manured with limited amounts of combined Nitrogen will not only enable us to meet some of the questions above suggested, but they will also prove whether or not the conditions of soil, atmosphere, temperature, &c., to which our experimental plants have been subjected were consistent with active and vigorous growth.

The fact of the evolution of Nitrogen in the decomposition of nitrogenous organic matter, illustrated in Sub-section D, p. 497 *et seq.*, indicated the danger of using such matter as a source of supply of Nitrogen. We have therefore used solutions of sulphate of ammonia (see Appendix, p. 542), by means of which we have been enabled to supply the plants with known quantities of combined Nitrogen at pleasure, as the progress of growth seemed to require.

In the following Table (XIV.) are given the numerical results of the experiments on the question of the assimilation of free Nitrogen in which the plants were supplied with combined Nitrogen beyond that contained in the seed sown. See also figs. 7, 8, 9, 10, 11, and 12, Plate XV., showing the character and extent of growth of six Graminaceous plants with extraneous supply of combined Nitrogen, corresponding to the six above them without such supply.

TABLE XIV.—Showing the Numerical Results of Experiments to determine whether Plants supplied with known and limited quantities of combined Nitrogen beyond that contained in the original Seed assimilate free Nitrogen.

General particulars of the Experiments.				Numerical particulars of the seed sown, and of the combined Nitrogen added.						Numerical results at the termination of the Experiment.					Summary.		
				Number of seeds.		Weights of the seed sown.	Nitrogen in seeds sown, and in manure.			Dry weights of Plants produced.	Nitrogen.				Nitrogen in Seeds sown, and in Manure.	Nitrogen in Total Products.	Gain or Loss of Combined Nitrogen.
Year, &c.	Description of Plant.†	Description of Soil or Matrix.	Period of Experiment.	Sown	That grew.	Fresh.	In seeds.	In manure.	Total.		In total Plant.	In Soil.	In Pot.	In condensed water.			
Graminaceæ.																	
						grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
1857	Wheat (6)	Prepared soil	May 16—Oct. 2	3	2	0·1830	0·1536‡	0·0040	0·0289	0·0329	6·835	0·0241	0·0133	0·0009	.....	0·0329	0·0383	+0·0054
	Wheat (7)	Prepared pumice	May 16—Sept. 20	3	3	0·1838	0·1543‡	0·0040	0·0289	0·0329	3·822	0·0213	0·0102	0·0016	.....	0·0329	0·0331	+0·0002
	Barley (8)	Prepared soil	May 20—Oct. 8	4	3	0·2160	0·1803	0·0037	0·0289	0·0326	3·034	0·0154	0·0171	0·0003	.....	0·0326	0·0328	+0·0002
	Barley (9)	Prepared pumice	May 20—Sept. 24	4	4	0·2178	0·1818	0·0037	0·0231	0·0268	4·401	0·0145	0·0171	0·0021	.....	0·0268	0·0337	+0·0069
1858	Wheat (9)	Prepared soil	April 27—Oct. 26	4	4	0·243	0·204	0·0040	0·0508	0·0548	7·31	0·0398	0·0138		traces.	0·0548	0·0536	−0·0012
	Barley (10)	Prepared soil	April 27—Oct. 26	4	2	0·190	0·158	0·0028	0·0468	0·0496	5·47	0·0349	0·0113		0·0002	0·0496	0·0464	−0·0032
	Oats (11)	Prepared soil	April 27—July 30	4	3	0·174	0·145	0·0032	0·0280	0·0312	1·204	0·0125	0·0040		0·0051	0·0312	0·0216	−0·0096
1858, A.*	Wheat	Prepared soil	June 29—Dec. 9	4	4	0·246	0·206	0·0040	0·0228	0·0268	3·82	0·0181	0·0092		0·0001	0·0268	0·0274	+0·0006
	Barley	Prepared soil	June 29— Dec. 9	4	3	0·194	0·162	0·0029	0·0228	0·0257	2·98	0·0160	0·0081		0·0001	0·0257	0·0242	−0·0015
	Oats	Prepared soil	June 29—Dec. 9	4	2	0·173	0·144	0·0032	0·0228	0·0260	1·28	0·0146	0·0051		0·0001	0·0260	0·0198	−0·0062
Leguminosæ.																	
1858	Pea (13)	Prepared soil	April 27—Aug. 24	3	3	0·642	0·539	0·0187	0·0040	0·0227	1·01	0·0114	0·0086		0·0011	0·0227	0·0211	−0·0016
	Clover (14)	Prepared soil	June 6—Oct. 26	...	...	0·5015	.....	0·0284	0·0428	0·0712	4·20	0·0313	0·0339		0·0013	0·0712	0·0665	−0·0047
1858, A.*	Bean	Prepared soil	June 29—Dec. 9	3	3	1·380	1·094	0·0523	0·0188	0·0711	4·30	0·0401	0·0253		0·0001	0·0711	0·0655	−0·0056
Other Plants.																	
1858	Buckwheat (16)	Prepared soil	Aug. 20—Nov. 22	42	24	1·0000	0·850	0·0200	0·0402	0·0308	1·97	0·0183	0·0101		0·0008	0·0308	0·0292	−0·0016

* These experiments were conducted in the apparatus of M. G. VILLE.

† The numbers given in brackets are those under which the respective plants are described in the "Abstract of the Records of Growth of the Plants" given in the Appendix, p. 548 *et seq.*

‡ The percentage of dry matter in the seed was not determined in these two cases; it is therefore assumed to be the same as in the wheat used in 1858, from which it would certainly not differ at all materially.

As in the case of the experiments already considered, so again with those to which the Table just given relates, it is seen, by reference to the last column, that there was a slight gain of Nitrogen in the experiments of 1857, but, almost without exception, a loss rather than a gain in those of 1858. Considering that there was a possible source of gain in 1857 in connexion with the slates used in that year (as explained below), and with the results of 1858 showing generally a loss rather than a gain when slates were not employed, we can interpret the whole in but one way.

In order to bring out fully the evidence afforded by these results of experiments in which the plants were supplied with more or less of combined Nitrogen during the progress of growth, we must consider them in three separate aspects :—

1. As regards the actual gain or loss of Nitrogen, as indicated by the figures given in the last column of the Table (XIV.).

2. As presented in the physiological evidence afforded during growth.

3. As exhibited on comparison with the experiments in which the plants had no other supply of combined Nitrogen than that of the original seed.

1. *The Numerical Results of Table XIV.*

Much that has been said with respect to the plants grown without extraneous supply of combined Nitrogen applies with equal force to those now under consideration ; and, so far as the evidence relating to the latter is of a different character, owing to the amount of combined Nitrogen at the disposal of the plants, it still is no more indicative of an assimilation of free Nitrogen than was that obtained with the plants grown without any artificial supply of combined Nitrogen.

In illustration of the probability that the slates used as lute-vessels were a source of Nitrogen to the plants grown in 1857, some of the observations made during growth should be adverted to. It is seen that the barley grown in pumice (1857) gives the largest gain of Nitrogen ; and it was observed that, soon after watering with the fluid drawn off from the surface of the slate, the pumice became covered with a slight coating of green matter. And nearly all the slates were found at the end of the experiment to have a slight coating of similar character beneath the pans in which the pots which contained the plants stood ; whilst, in the experiments of 1858, when glazed earthenware lute-vessels were employed, no such phenomenon was observed.

The slight loss of Nitrogen exhibited in the experiments of 1858 is easily accounted for on a consideration of the conditions involved. With regard to the peas, clover, and beans, the physiological circumstances of growth detailed in the Appendix, taken in connexion with the evidence that has been adduced as to the loss of Nitrogen during the decomposition of nitrogenous organic matter, must be supposed to explain the loss in their case, as in some of the experiments in which no extraneous supply of combined Nitrogen was employed.

The loss of Nitrogen indicated in the cases of the wheat, barley, oats, and buck-

wheat (1858) would not be so easily explained, had not the Nitrogen in the drain-water remaining at the end of the experiment been determined. Our object in doing this was twofold :—

1. To ascertain whether the luting at the bottom of the shade had allowed rain-water to pass, thus affording a source of combined Nitrogen to the plants.

2. To see if the plants growing in soil to which combined Nitrogen was added, had evolved any ammonia.

It was, of course, not possible to accomplish both these purposes. But the fact that ammonia was found in the condensed water only in the cases where there was a *loss* in the total quantity of combined Nitrogen would lead to the inference that both the presence of ammonia in this water, and the loss of combined Nitrogen in the experiment, were due to the same cause.

The condensed water showing the amount of combined Nitrogen recorded in the Table (XIV.) was that which had been evaporated and condensed during the last four weeks of growth (1858) ; and during this period the high temperature, and the advanced stage of the plants, were favourable to the evaporation of ammoniacal water. A considerable part would condense on the interior of the shade, owing to its comparatively low temperature ; but a certain quantity of that which was in the state of vapour during the passage of the air through the apparatus would be borne forward into the sulphuric acid in the bulb-apparatus M, and thus occasion a loss in the amount of combined Nitrogen determined in connexion with the plants. The reason why the loss is greater with the oats (as it is in both experiments) than with the other cereals is not perfectly clear ; but the circumstances of growth seemed to afford some explanation of the fact. In one case, at least, they ripened at a much warmer period of the season, and they became much drier in stem and leaf, and were therefore more liable to evolve ammonia. On these points, the circumstances of growth detailed in the Appendix should be consulted.

In considering the column of gain or loss of Nitrogen, it is very desirable to take into account the total quantity of Nitrogen at the disposal of the plant, in the different series of experiments. It is also important to consider the amount of growth in the experiments made under the different conditions. The following Tables (XV. and XVI.) bring out the character of the results in these respects more clearly than they can be gathered from Tables XII. and XIV. Table XV. shows, for the plants grown without supply of combined Nitrogen beyond that contained in the seed, and Table XVI. for those grown with such supply, the dry matter, and the Nitrogen, per seed sown,—the dry matter, and the Nitrogen, in the total produce of each seed that grew,—and the per cent. of the total Nitrogen at the disposal of the plant which it appropriated. Finally, the last two columns of Table XVI. show the amounts of dry matter, and of Nitrogen, in the produce grown with the extraneous supply of combined Nitrogen, in relation to those in the produce grown without such supply.

Table XV.

General particulars of the Experiments.			Number of Seeds.		Dry Matter.			Nitrogen.			
Year, &c.	Description of Plants.	Description of Soil or Matrix.	Sown.	That grew.	Per seed sown.	In the Produce, per seed that grew.	In Produce, that in seed taken as 1.	Per seed sown.	In the Produce, per seed that grew.	In Produce, that in seed taken as 100.	Per cent. in Dry Produce.
					Graminaceæ.						
					grm.	grm.		grm.	grm.		
1857 ...	Wheat	Prepared soil	6	5	0·0511	0·2824	.....	0·00133	0·00144	108·3†	0·51
	Barley	Prepared soil	6	6	0·0449	0·1350	3·01	0·00093	0·00078	83·9	0·58
	Barley	Prepared pumice	6	6	0·0449	0·1542	3·43	0·00093	0·00075	80·6	0·48
1858 ...	Wheat	Prepared soil	8	8	0·0504	0·2175	4·31	0·00098	0·00070	71·4	0·32
	Barley	Prepared soil	8	6	0·0403	0·0933	2·31	0·00071	0·00052	73·2	0·54
	Oats	Prepared soil	8	8	0·0357	0·1435	4·02	0·00080	0·000525	65·6	0·36
1858,A.*	Wheat	Prepared soil	8	7	0·0504	0·15143	3·00	0·00098	0·00058	59·2	0·38
	Barley	Prepared soil	8	8	0·0401	0·0888					
	Oats	Prepared soil	8	7	0·0360	0·09857	2·74	0·00080	0·00054	67·5	0·55
					Leguminosæ.						
1857	Bean	Prepared soil	2	2	0·7492	3·514	4·69	0·03980	0·03145	79·0	0·89
1858 ...	Bean	Prepared soil	3	3	0·4940	1·6250	3·29	0·02500	0·0245	98·0	1·51
	Pea	Prepared soil	3	3	0·1802	0·3233	1·79	0·00630	0·0034	54·0	1·05
					Other Plants.						
1858	Buckwheat	Prepared soil	24	13	...	0·03461	.....	0·00083	0·00054	65·1	1·56

Table XVI.

General particulars of the Experiments.			Number of Seeds.		Dry Matter.			Nitrogen.				Relation of Produce per seed with Ammonia, to that without it taken as 1.	
Year, &c.	Description of Plants.	Description of Soil or Matrix.	Sown.	That grew.	Per seed sown.	In Produce, per seed that grew.	In Produce, that in seed taken as 1.	Per seed sown.	In the Produce, per seed that grew	In Produce, that in seed and manure taken as 100.	Per cent. in Dry Produce.	Dry Matter.	Nitrogen.
					Graminaceæ.								
					grm.	grm.		grm.	grm.				
1857 ...	Wheat	Prepared soil	3	2	0·0512	3·4175	66·75	0·00133	0·01205	73·2	0·35	12·10	8·36
	Wheat	Prepared pumice	3	3	0·0514	1·2740	24·79	0·00133	0·00710	64·7	0·55	4·51	4·93
	Barley	Prepared soil	4	3	0·0451	1·0113	22·42	0·00092	0·00513	47·2	0·51	7·49	6·58
	Barley	Prepared pumice	4	4	0·0455	1·1002	24·18	0·00092	0·00362	54·1	0·33	7·13	4·83
1858 ...	Wheat	Prepared soil	4	4	0·0510	1·8275	35·83	0·00103	0·00995	72·8	0·54	8·40	14·21
	Barley	Prepared soil	4	2	0·0395	2·7350	69·24	0·00070	0·01745	70·5	0·64	29·31	34·21
	Oats	Prepared soil	4	3	0·0362	0·4013	11·08	0·00080	0·00416	45·9	1·04	2·80	7·92
1858,A.*	Wheat	Prepared soil	4	4	0·0515	0·9550	18·54	0·00100	0·00452	67·5	0·47	6·31	7·79
	Barley	Prepared soil	4	3	0·0405	0·9933	24·52	0·00072	0·00533	62·2	0·54		
	Oats	Prepared soil	4	2	0·0360	0·6400	17·78	0·00080	0·00730	56·1	1·14	6·49	13·52
					Leguminosæ.								
1858 ...	Pea	Prepared soil	3	3	0·1797	0·3366	1·87	0·00623	0·00380	50·2	1·13		
	Clover	Prepared soil	...	...	...	.....	.....	...	...	44·0			
1858,A.*...	Bean	Prepared soil	3	3	0·3646	1·4333	3·93	0·01743	0·01337	56·4	0·93		
					Other Plants.								
1858	Buckwheat	Prepared soil	42	24	0·0202	0·0821	4·06	0·00047	0·00076	57·6	0·92	2·37	1·41

* These experiments were conducted in the apparatus of M. G. Ville.

† There is here evidence that a part of the Nitrogen of the seed that did not grow was appropriated by the plants growing from the other seeds.

There are several obvious inferences to be drawn from the figures in these Tables. To some we shall refer further on, in the proper order of the discussion. We here simply call attention to the very great increase of growth when an extraneous supply of combined Nitrogen was provided, as exhibited in the last two columns of Table XVI.

2. *Consideration of the Physiological Evidence as bearing upon the question of the assimilation of free Nitrogen.*

However directly the quantitative details given in the Tables may bear upon the question at issue, it is very important to consider them in connexion with the physiological details of the experiments. In order to estimate the value of the evidence afforded in this particular, the indications manifested from the earliest period of growth should be noticed.

Reference to the Notes of the progress of the plants, given in the Appendix, will show that all the plants when they first came up looked green and vigorous, indicative of their being at that period in circumstances embracing all the conditions essential to healthy growth. As already pointed out, they at that time were probably supplied with an excess of combined Nitrogen in relation to their immediate wants. After some days, varying with the nature of the plants, they began to lose their deep-green colour, and to assume a lighter-green, or pale-yellow tint, indicative of a want of combined Nitrogen. We have already pointed out how favourable, probably, would be the conditions here afforded for the assimilation of free Nitrogen, when the plant was passing from the state in which it had an excess to that in which it had a deficiency of combined Nitrogen for the demands of growth. The vigorous development of the plants grown in garden soil, but under the same conditions as to atmosphere, &c. as the other experimental plants, indicates that the conditions of atmosphere provided in the experiments were not at fault (see Appendix, Experiments Nos. 12, 1857, and 15, 1858 ; also fig. 13, Plate XV.). In order to test whether the sum of all the conditions, excepting those connected with a sufficient supply of combined Nitrogen, were appropriate for vigorous growth, we have only to provide some combined Nitrogen when the plants show the declining vigour just described ; and if this be all they require, they will resume their healthy green colour. Or if we add the combined Nitrogen before the plants arrive at the period in question, it will prevent them assuming the pale-green or yellow colour. We have had recourse to both of these expedients ; and each, so far as the Cereals, buckwheat, and clover are concerned, has yielded a result indicating that all the conditions of the experiments, excepting those connected with a sufficient supply of combined Nitrogen, were adapted for healthy growth.

The plants to which ammonia was given in 1857, were allowed to suffer more before they received it than those of 1858 ; yet in thirty-six hours after the addition of combined Nitrogen to the soil, in amount not exceeding $1\frac{1}{2}$ milligramme of the element to each plant, they began to manifest an improved appearance. In two or three days the improvement was quite marked ; but at the termination of periods varying from nine to

eighteen days, the plants seemed to have consumed all the combined Nitrogen supplied to them—or rather all of it that had not become inaccessible to them in the soil. They then began to manifest the same indications of defective supply as before. Plants so circumstanced must therefore, at a more advanced stage of growth than before they had been supplied with ammonia, have passed from a point at which they had an excess of combined Nitrogen, to that in which they had an insufficiency. They must hence, again, have been subjected to those conditions which we have assumed to be probably very favourable to the assimilation of free Nitrogen.

Reference to the details of growth given in the Appendix will show that several times during the progress of the plants the above phenomena were manifested. A new increment of combined Nitrogen caused a new increment of growth, a greener colour, and a more vigorous appearance generally. This was soon followed by the recurrence of the pale colour. In some instances, more ammonia was not supplied until the plants seemed almost past recovery: in a few cases they were quite so. The addition of ammonia now (excepting in the few cases just referred to) produced a revivification, to be followed in a short time by the indications of some want, and so on.

A considerable range of conditions of growth was thus provided. Just after each addition of combined Nitrogen the plants must have been supplied with an excess of this element in an available form. The evidence of this was afforded in the obviously increased means of consumption, evinced in the formation of new shoots from the base of the plants, or from their nodes. But these new shoots were too vigorous to allow the plants to go on long without suffering for want of a new supply of combined Nitrogen. In passing to this point, the newly-formed and vigorously-growing portion of the vegetable matter would be in the condition we have assumed to be the most favourable for assimilating free Nitrogen. Instead of doing this, however, it soon began to suffer, and continued to do so until a new supply of combined Nitrogen was added, when new vigour succeeded, to be followed again shortly by a cessation of growth. This cycle of conditions, repeated several times during the growth of the same plant, and the experiment similarly conducted with a number of pots of plants of different kinds, with like results in all the cases, affords a wide range of circumstances such as we have assumed to be favourable to the assimilation of free Nitrogen; but such an assimilation has not taken place.

Without the physiological details, it might not have been clear that the plant had not an excess of combined Nitrogen at its disposal during the greater period of its growth after the addition of the artificial supplies of it, since a considerable proportion of that added remained in the soil at the termination of the experiments, as Tables XIV. and XVI. show. But it is not difficult to imagine that a few milligrammes of ammonia intermingled with 1,500 or 1,600 grammes of soil (and pot), might become distributed over such an extent of surface, and be so completely absorbed, as that a considerable proportion should remain inaccessible to the plant. The physiological evidence leaves no doubt this was the case.

The Graminaceous plants of the experiments of 1858 were supplied with a considerable quantity of combined Nitrogen at an earlier period of growth than those of 1857 (see Tables showing the dates of addition, Appendix, pp. 542, 543), and they were not allowed to exhibit such marked signs of decline of vigour before receiving their fresh supplies. There is, however, no marked distinction in the proportion of the total supply appropriated by the plants, and left in the soil, respectively, in the two cases.

The Graminaceæ under the title of "1858, A" (those grown in M. G. Ville's case) were treated similarly to the others of 1858, excepting that the combined Nitrogen was given to them at an earlier period of their growth, and they were not allowed to suffer at any time for want of it. We shall notice the difference in result presently.

In addition to the evidence of the physiological phenomena as bearing upon the amount of growth due to the supply of ammonia, attention should be called to the remarkable character of growth which was manifested. The evidence afforded on this head, is of interest in considering the question of the character of the conditions most favourable to the assimilation of free Nitrogen; and it also brings to view some remarkable features in vegetable physiology.

It will be seen, by reference to the Notes in the Appendix, that, shortly after the addition of ammonia for the first time to the Graminaceæ (1857 and 1858), the plants began to throw out new shoots at the base of the principal stem. It would thus appear that the plant, being supplied at the commencement of its growth with only the limited quantity of combined Nitrogen contained in its seed, had developed a stem commensurate with that quantity. But when new quantities of combined Nitrogen were placed at the disposal of the plant, forces were thus called into activity which were greater than could operate through the medium of the original stem. Some of the new shoots have come forth close to the surface of the soil, some at the first, and some at the second nodes. The character of growth in this respect can be best studied by reference to the drawings of the plants given in Plate XV.

Another and no less remarkable feature was the formation of roots at the second and third nodes above the ground in the case of most of the Graminaceous plants to which ammonia-salt was added as manure (see Plate XV.). These roots came out around the node, and extended downwards—several of them reaching the soil from heights varying from $\frac{1}{2}$ to $1\frac{1}{2}$, or even 2 inches, and penetrating it to the bottom of the pot. The most marked instance of this kind of growth was that of the barley represented in fig. 11, Plate XV., and in more detail, with special reference to the points now under consideration, in fig. 16, Plate XV. As will be seen in the figures, roots and new stems come from the same node, making the latter a veritable starting-point, or new axis of growth, like the seed in the first instance. The original stems, below these nodes, did not increase much in size beyond what they had attained before the addition of ammonia; but the stems above the nodes became much larger than the portions below them, as also did those of the new shoots.

Finally, so long as the conditions of growth of the plants were such that an addi-

tional supply of combined Nitrogen would cause increased development, so long must the physiological conditions have been such as to require available Nitrogen, and they must therefore have been more or less favourable to the assimilation of free Nitrogen, provided such assimilation were possible. Hence, the fact that this did not take place under the circumstances which have been described, seems to show that, at least in the case of these Graminaceæ, it is not possible.

Some of the remarks which we have made with regard to the influence of a supply of combined Nitrogen upon the growth of the Graminaceæ, apply also, in a greater or less degree, to the other plants experimented upon. We shall not comment here in detail upon the value of each experiment, but simply call attention to the columns of gain or loss of Nitrogen, in the Tables, and to the notes in the Appendix indicating the circumstances of growth of the plants.

With regard to the Leguminosæ experimented upon, it is to be observed that the development was by no means so satisfactory as in the case of the Graminaceæ. Hence the evidence which the results relating to them afford against the fact of assimilation of free Nitrogen must be admitted to apply to a more limited range of conditions of growth, and, therefore, to be less conclusive against the possibility of such assimilation. Still, so far as they go, the results with these plants, and also those with buckwheat, tend to confirm those obtained under the more favourable circumstances of growth with the cereals. It will be remembered, however, that M. BOUSSINGAULT experimented with a great many Leguminous plants, and generally succeeded in getting much more healthy growth than we were able to do in the cases to which the figures in the Tables refer. Yet in no case did he find any such gain of Nitrogen as to lead him to the conclusion that these plants, any more than the Graminaceæ, assimilated free or uncombined Nitrogen. Our own experiments with Leguminous plants are, however, not yet concluded; so that we hope to supply some additional evidence on this subject, on a future occasion.

Relations of the Plants grown with a supply of ammonia to those grown without it.

We have already called attention to the fact that the physiological phenomena exhibited in the progress of the plants grown under the two different conditions as regards the supply of combined Nitrogen at their disposal, afford satisfactory evidence that the conditions provided in soil and atmosphere were all that were requisite in experiments for the solution of the question at issue with regard to the Cereals. The great development of these plants when ammonia was supplied (which was in fact almost in proportion to the amount supplied), the cessation of growth with the limit of the supply, together with the contrast between the growth with the aid of the ammonia and that without it, all afford evidence in one direction in regard to the question at issue, so far as these plants are concerned.

In Table XIV., relating to the plants to which ammonia was supplied, an experiment with clover is recorded. Reference to the remarks in the Appendix, p. 573, will show

that we failed to get any growth with clover without the addition of ammonia. Hence, excepting so far as this fact is itself a point for remark, no contrast can be drawn between the growth of this plant with and without an extraneous supply of combined Nitrogen.

From what has already been said, it will be easily understood that the contrast between the beans and peas grown with and without the addition of ammonia is not very satisfactory. These plants proved to be so sensitive, under the conditions provided in the experiments, that it was obvious that, in many cases, they suffered from other causes than a want of combined Nitrogen, which we were not able to control. In but one experiment with such plants, that with the bean "1858, A.' (Table XIV.), was the influence of a supply of combined Nitrogen so marked as to indicate that the plants were previously suffering for want of such supply. It will be seen, by reference to the Table, that, in the case here referred to, the seeds sown contained 0·0523 gramme of Nitrogen, and that 0·0188 gramme was added in the form of ammonia-salt—making in all 0·0711 gramme of combined Nitrogen involved in the experiment. Of this the plants appropriated 0·0401 gramme—about one-fifth less, therefore, than was supplied in the seeds alone. Yet, although the numerical results, taken by themselves, thus afford but little evidence of the effect of the 0·0188 gramme of Nitrogen added in the form of ammonia, the increased vigour of growth on the addition did afford such evidence. In contrast with this single result, however, attention may be called to the results with the beans grown without any other supply of combined Nitrogen than that contained in the seed sown. The bean plants so grown in 1857, appropriated nearly four-fifths of the Nitrogen of their seed; and those grown in a similar way in 1858, appropriated a considerably larger proportion of the combined Nitrogen so provided to them.

From a review of the whole of the results considered in this Section, it appears, then, that in the case of the Graminaceous plants experimented upon the growth was the most healthy, and such as provided a wide range of conditions for the assimilation of free Nitrogen, provided this were at all possible. The growth of the Leguminous plants was not so healthy, and did not, therefore, provide such a wide range of conditions for the possible assimilation of free Nitrogen. Nor was the growth of other plants so satisfactory as that of the Graminaceous ones. In all, the growth was more or less increased by the supply of combined Nitrogen beyond that contained in the seed. The effect of such supply was the most marked with the Graminaceous plants—the increase in the produce of dry vegetable substance due to extraneous supply of combined Nitrogen being, in their case, eight, twelve, and even nearly thirty-fold, according to the amount of Nitrogen so provided. Yet, with nineteen experiments with Graminaceous plants, six with Leguminous ones, and some with plants of other descriptions—with such great variation in the amount and character of growth in the several cases—and with such great variation in the amount of combined Nitrogen involved in the experiments, in

no case have the results been such as to lead to the conclusion that there was an assimilation of free, or uncombined, Nitrogen.

The results of the whole inquiry may be very briefly enumerated as follow :—

The yield of Nitrogen in the vegetation over a given area of land, within a given time, especially in the case of Leguminous crops, is not satisfactorily explained by reference to the hitherto quantitatively determined periodical supplies of *combined* Nitrogen.

Numerous experiments have been made by M. Boussingault, from which he concludes that free or uncombined Nitrogen is not a direct source of the Nitrogen of vegetation. M. G. Ville, on the other hand, concludes, from his results, that free Nitrogen may be a source of a considerable proportion of the Nitrogen of growing plants. The views, or explanations, of other experimenters, on this disputed point, are various, and inconclusive.

It was found that the conditions of growth adopted in our own experiments on the question of the assimilation of free Nitrogen by plants, were consistent with the healthy development of various Graminaceous plants, but not so much so for that of the Leguminous plants experimented upon.

From the results of various investigations, as well as from other considerations, we think it may be concluded that, under the circumstances of our experiments on the question of the assimilation of free Nitrogen by plants, there would not be any supply to them of an unaccounted quantity of combined Nitrogen, due either to the formation of oxygen-compounds of it under the influence of ozone, or to that of ammonia under the influence of nascent hydrogen.

We have found that free Nitrogen is given off in the decomposition of nitrogenous organic matter, under certain circumstances. But, considering the circumstances of such evolution, and those to which the nitrogenous organic matter necessarily involved in experiments on the question of the assimilation of free Nitrogen by plants is subjected, it may, we think, be concluded that there would be no loss of combined Nitrogen from this cause in such an experiment, excepting in certain cases, when it might be presupposed.

Our experimental evidence, so far as it goes, does not favour the supposition that there would be any loss of combined Nitrogen in our experiments on the question of assimilation, due to the evolution of free Nitrogen from the nitrogenous constituents of the plants during growth.

In numerous experiments with Graminaceous plants, grown both with and without a supply of combined Nitrogen beyond that contained in the seed sown, in which there was great variation in the amount of combined Nitrogen involved, and a wide range in the conditions, character, and amount of growth, we have in no case found any evidence of an assimilation of free or uncombined Nitrogen.

In our experiments with Leguminous plants the growth was less satisfactory; and

the range of conditions possibly favourable for the assimilation of free Nitrogen was, therefore, more limited. But the results recorded with these plants, so far as they go, do not indicate any assimilation of free Nitrogen. Since, however, in practice, Leguminous crops assimilate, from some source, so very much more Nitrogen than Graminaceous ones, under ostensibly equal circumstances of supply of combined Nitrogen, it is desirable that the evidence of further experiments with these plants, under conditions of more healthy growth, should be obtained.

Results obtained with some other plants are in the same sense as those obtained with Graminaceæ and Leguminosæ, in regard to the question of the assimilation of free Nitrogen.

In view of the evidence afforded of the non-assimilation of free Nitrogen by plants under the wide range of circumstances provided in the experiments, it is desirable that the several actual or possible sources of *combined* Nitrogen to plants should be more fully investigated, both qualitatively and quantitatively.

If it be established that the processes of vegetation do not bring free Nitrogen into combination, it still remains not very obvious to what actions a large proportion of the existing combined Nitrogen may be attributed.

Appendix.

Received subsequently to the reading of the paper.

A.—*Preparation of solutions for manuring the Plants, dates of application, and quantities applied.*

Sulphate-of-Ammonia solution.—Ordinary ammonia-water was distilled from a flask, the vapour condensed in a receiver containing pure distilled water, and the strength of the solution determined by the volumetric method, by means of dilute sulphuric acid of known strength, the preparation of which is described further on, at p. 545. A given volume of the ammoniacal liquid thus prepared was neutralized by pure dilute sulphuric acid, of which the quantity added was determined by measurement, and the strength of the solution calculated accordingly. It was intended that each cubic centimetre should supply about one-tenth of a milligramme of combined nitrogen. The exact strength of the sulphate-of-ammonia solutions used in the course of the experiments was as under :—

Table I.

When used.	Volume of the pipette measure employed.	Combined nitrogen in a pipette measure of the solution.
	septems*	gramme.
In the experiments of 1857	111·2	0·00578
In the experiments of 1858, to August 10 inclusive	100·0	0·004
In the experiments of 1858, after August 10	100·0	0·00359

Tables II. and III. show the dates of the application of the above solutions to the different plants, and the amounts of nitrogen so supplied.

Table II.—Showing the supply of combined Nitrogen, as Sulphate-of-Ammonia solution, to plants grown in 1857.

Dates.	Nitrogen supplied.			
	Wheat, in prepared soil.	Wheat, in prepared pumice.	Barley, in prepared soil.	Barley, in prepared pumice.
	gramme.	gramme.	gramme.	gramme.
June 10	·00578	·00578	·00578	·00578
July 4	·00578	·00578	·00578	·00578
July 11	·00578	·00578	·00578	·00578
July 22	·00578	·00578	·00578	·00578
July 29	·00578	·00578	·00578	
Total	·02890	·02890	·02890	·02312

* A septem measure is that of 7 grains ($=\frac{1}{1000}$ of a pound avoirdupois) of water; that is, rather less than half a cubic centimetre, which is equal to 15·43235 grains (or 1 gramme) of water.

TABLE III.—Showing the supply of combined Nitrogen, as Sulphate-of-Ammonia solution, to plants grown in 1858.

Dates.	Nitrogen supplied.									
	Wheat.	Barley.	Oats.	Wheat*.	Barley*.	Oats*.	Pea.	Clover.	Bean*.	Buck-wheat.
	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
May 22	·0040	·0040	·0040							
June 7	·0040	·0040	·0040	.....	.....	.....	·0040	·0040		
June 21	·0040	·0040	·0040							
June 26	·0040	·0040	·0040	.....	.....	.....	.....	·0040		
July 3	·0040	·0040	·0040	.....	.....	.....	.....	·0040		
July 12	·0040	·0040	·0040	.....	.....	.....	.....	·0040		
July 14	·0040	·0040	·0040	·0040	·0040	·0040	.....	·0040	·0040	
July 19	·0040	·0040	.....	·0040	·0040	·0040	.....	·0040	·0040	
July 28	.....	·0040	.....	·0040	·0040	·0040	.....	·0040		
July 29	·0040									
August 10	·0040	.....	.....	.....	.....	.....	.....	·0040		
August 17	·0036	·0036	.....	·0036	·0036	·0036	.....	·0036	·0036	
August 24	.....	·0036								
August 26	·0036									
September 7	·0036	·0036	.....	·0036	·0036	·0036	.....	·0036	·0036	·0036
October 5	.....	.....	.....	.....	.....	.....	.....	·0036	.....	·0036
October 24	.....	.....	.....	·0036	·0036	·0036	.....	.....	·0036	·0036
Total	·0508	·0468	·0280	·0228	·0228	·0228	·0040	·0428	·0188	·0108

Phosphate-of-Soda solution.—The strength of a dilute solution of phosphoric acid was determined by means of a titrated alkali-solution (for the preparation of which see page 545) ; and it was then neutralized by carbonate of soda. Each pipette measure of this solution given to the plants supplied about ·01 gramme phosphate of soda. It was only employed in the experiments of 1858. In the records of growth of the plants, it is stated whenever they were manured with this solution.

Sulphuric-Acid solution.—The strength of some very dilute pure sulphuric acid was determined in the same manner as was that of the phosphoric acid, as stated above. I_t was then so far reduced, that the pipette measure by which it was applied to the plants contained exactly as much SO_3 as the pipette of sulphate-of-ammonia solution then in use, namely, ·0114 gramme SO_3, corresponding to ·004 gramme N. For the application of this solution see the records of growth of the plants.

The value of each of the above solutions was determined by analysis, to ensure that it was such as was supposed.

B.—*Taking up the Plants, preparation for analysis, methods of analysis, &c.*

At the termination of growth the glass shade was washed outside, quicksilver was poured into the groove to displace from it the condensed water not removable by the arrangement of apparatus of 1857, or already collected in the drain-water bottle adopted in that of 1858, as the case might be, and the shade was then removed. The

* These plants were grown in M. G. VILLE'S case.

previously covered portions of the slate or stone-ware lute were then washed with pure distilled water, and the wash-water was added to the condensed or drain-water. In the experiments of 1858 this fluid was analysed separately, but in those of 1857 it was mixed and dried down with the soil.

The pot, with the soil and plants, was removed to a clean table covered with white paper, the plants measured in all their parts and then cut off at the surface of the soil; the roots were removed, slightly washed from soil, and observed. The plants were then put into a small wide-mouthed bottle, generally stem and root together, but sometimes they were put into separate bottles. In the experiments of 1857 the contents of the bottles were dried in a water-bath, with a current of air, previously washed through sulphuric acid, passing through the bottle and thence through a solution of a known quantity of pure oxalic acid. But it was found that no appreciable amount of ammonia was thus accumulated. Hence, in 1858, a little oxalic acid (in solution) was added to the vegetable matter, and the whole dried in the water-bath without the above precaution.

When dry, the vegetable matter was cut small by means of a pair of clean long scissors, reaching to the bottom of the bottle. In this way the substance was reduced to a considerable degree of fineness, and it was still further ground up in the mortar when mixed with soda-lime for analysis. When duplicate analyses were to be made, the matter was carefully divided so as to ensure equal proportions of stem, fine leafy matter, &c., in each half. Hence, if both analyses were successfully conducted, the results were mutually confirmatory; or if one portion were lost, the other still represented a proportionate amount of the whole material.

The *soil* was removed from the pot to a porcelain dish, and a sufficient amount of a solution of oxalic acid added to keep it acid. The mixture was then heated on a sand-bath (stirring constantly) until most of the water was expelled, more fully dried in a water-bath, and then preserved in well-corked bottles for analysis. The pots were pounded up; those of 1857 being preserved and analysed separately, and those of 1858 mixed with the soil before it was dried with oxalic acid. The pieces of flint at the bottom of the pot were also pounded and mixed with the soil.

For analysis, 150 to 200 grammes of the soil, pot, or mixture, were mixed with about half the volume of soda-lime, the whole put into a large combustion-tube, some soda-lime put in in advance of the mixture, and then asbestos, as usual. The combustions were made in charcoal furnaces, and the ammonia collected in titrated sulphuric acid, of which the strength, and the amounts employed, are described at pp. 545, 546. When very small quantities of nitrogen were involved, the ammonia from two or three tubes of substance was sometimes collected in the same quantity of acid, so as to diminish the error of titration. It was found, however, to be better to use very small quantities of acid, and to estimate the product of each combustion separately; for, by the former method, if any accident occurred in the second or third combustion, it involved the loss of the determination of the products previously collected.

Preparation of the titrated solutions.

A weighed quantity of pure, dry carbonate of soda was dissolved in water, and to the solution water added to a given volume. As a preliminary step, the strength of some dilute sulphuric acid was tested against a given volume of the carbonate-of-soda solution; and from the data thus obtained, by further dilution a large quantity of acid was made of about the strength desired. The exact value of this acid was then ascertained by repeated trials with the standard carbonate-of-soda solution. To accomplish this, a given volume of the soda-solution was put into a beaker, a little litmus added, and the mixture heated over a spirit-lamp. The acid to be tested was then allowed to flow from a burette until a wine-red colour (indicating that the carbonate is converted into sulphate and bicarbonate with carbonic acid in solution) was produced. On boiling, the blue colour is restored; acid is added until red; the boiling is repeated, till the blue returns; acid again added, and so on, until the solution remains red on the addition of the last drop. The point at which the permanent change takes place in the first trial being known, the experiment is easily repeated so as to ensure great accuracy.

Thus, 50 septems of a solution of carbonate of soda of which 1000 septems contained 6·652 grammes of the salt, required, for neutralization as above, the following number of septems of the dilute acid, in six different trials—

58·3, 58·2, 58·3, 58·3, 58·2, 58·2; mean 58·25.

Hence—

$$\frac{6{\cdot}652}{1000}\times\frac{50}{58{\cdot}25}\times\frac{N}{NaO,\,CO_2}=\frac{6{\cdot}652}{1000}\times\frac{50}{58{\cdot}25}\times\frac{14}{52{\cdot}98}=0{\cdot}001508 \text{ gramme N.}$$

The mean of six experiments with a solution of carbonate of soda of another strength gave in the same way 0·0015008 gramme N; and we adopted the mean, or 0·001504 gramme, as the amount corresponding to one septem of the titrated acid.

It remained to prepare an alkaline solution to test against this standard acid. At first a solution of sugar-lime was employed; but this being found to be liable to constant change, due doubtless to fermentation, a solution of caustic soda was had recourse to. This solution was prepared of such dilution that the extreme error possible in reading off a unit of volume on the burette should be much less than would be admissible as the maximum error of analysis. The burette was of small enough diameter to allow of one-tenth of a septem being read off on it; and the alkali-solution was so dilute that it required about three septems of it to neutralize one septem of the titrated acid. Hence one septem of the alkali-solution corresponded to only about one-half of a milligramme of nitrogen, and the probable error of reading would therefore amount to only about one-twentieth of a milligramme.

In the case of the sugar-lime solution, it was found necessary to test its strength against that of the acid every day that it was employed. But the soda-solution, if properly prepared, and well preserved, remained for months unchanged; so that, when its value was once established against that of the standard acid, it could be expressed

by a number of four or five digits, which, multiplied by the number of septems of alkali representing the product in an analysis, gave the actual quantity, in grammes, of the nitrogen to be estimated.

Amount, and measurement, of the titrated acid used in Nitrogen determinations.

It was desirable that at least three times as much acid should be used as would be neutralized by the ammonia formed. The acid being more concentrated than the alkali, it required a more exact method of measurement than was afforded by the burette used for the latter. Pipettes, of which the diameter at the point of reading off is comparatively small, and which hence admit of a higher degree of accuracy, were therefore employed. In the construction of those to be used, care was taken to maintain the same relation of the diameter of the neck at the point of reading to the entire volume in instruments of different sizes—a condition seldom observed by makers of pipettes. When the quantity of nitrogen involved in an analysis was very small—as in the case of the soils and pots in the experiments without nitrogenous manure—only about six septems of the titrated acid, measured in a small pipette with a very narrow neck, were used. The exact volume of the pipette-ful of acid was not a matter of any consequence. It was only essential to ascertain its exact value expressed in septems of the titrated alkali-solution. When the amount of nitrogen involved was larger, and more under control—as for example when grains were to be analysed—care was taken to operate on such a quantity of nitrogenous material that the number of septems of the alkali representing its nitrogen should be sufficiently large to render the constant errors of titrating, reading, &c., inappreciable. This end was attained when the substance experimented upon contained 5 to 8 milligrammes, or more, of nitrogen.

Combustion-tubes, bulbs, &c.

The combustion-tubes used in the determinations of nitrogen in the soils, pots, &c., were about 3 feet long and about 1 inch in diameter. The bulb-apparatus was capable of holding two-and-a-half to three times as much fluid as that usually employed; but the central and lowest bulb, and particularly its tubular connexions with the other bulbs, were very small, so that a small quantity of liquid could close the passage. This arrangement was necessary owing to the small quantity of acid frequently used, and the large amount of water driven off in the combustion from the large quantities of soil and soda-lime. For the combustion of the experimentally grown plants smaller tubes were employed; and for seeds, &c., ordinary combustion-tubing was used.

The Soda-lime.

Before use, the soda-lime was ignited with 2 per cent. of pure sugar, in order to ensure its freedom from ammonia-yielding matter. It was then slaked with pure distilled water, dried, and kept in well-corked bottles.

Accuracy of the method for the determination of nitrogen by combustion with soda-lime, &c.

In order to ascertain the accuracy of the method before relying upon it for the purposes of the investigation, a few preliminary experiments were made upon the determination of small and known quantities of nitrogen, mixed with large quantities of soil, which had been previously freed from combined nitrogen as in the preparation of the soils for the plant-experiments. The nitrogenous substance taken for the purpose was the powdered crystals of purified quadroxalate of ammonia, $\left\{\begin{matrix}NH_3\\H\end{matrix}\right\}O,\ (C_2\ O_3)_4+7HO$. The results were as follow—

Experiment 1.—50 grammes of the prepared soil were mixed with quadroxalate containing by calculation 0·0024 gramme nitrogen ; and on burning with soda-lime, and determining as above described, 0·0027 gramme nitrogen was found.

Experiment 2.—100 grammes of the soil mixed with quadroxalate equal, by calculation, to 0·0035 gramme nitrogen, gave on combustion 0·0037 gramme nitrogen.

The error of analysis was, therefore, three-tenths of a milligramme of nitrogen with the 50 grammes, and two-tenths with the 100 grammes of soil. These results were obtained at the commencement of the inquiry, with comparatively large quantities of titrated acid, and therefore before experience had suggested the precautions to be adopted to reduce the errors of determination to the minimum. They may hence be taken as examples of the maximum errors of analysis, but they are less than would affect the bearing of the results in the investigation on the question of assimilation.

Testing for Nitric acid.

The indigo test, as recently refined by Boussingault*, and the protosulphate-of-iron test, were both employed. When nitric acid was sought for and not found, if practicable the negative result was always confirmed by the addition to some of the substance under examination of a quantity of nitric acid (in the form of nitrate) less than could affect any conclusions to be drawn from the fact of its presence or absence in the substance in question. In all the cases of such addition the re-examination showed the presence of nitric acid.

The method of Boussingault was much more delicate than the protosulphate-of-iron test ; but, on the other hand, the latter was much less liable to give deceptive indications, dependent on other circumstances than the presence of nitric acid. In using the protosulphate test, the aqueous extract of the substance under examination was evaporated to a small volume with excess of fixed alkali, then transferred to a test-tube, and further evaporated till only a few drops remained. A considerable excess of concentrated sulphuric acid was then added, and on the surface of the liquid a concentrated solution of protosulphate of iron was carefully poured without agitation, by means of a small pipette with a mouth of almost capillary fineness. The characteristic brown tinge indicated the presence of nitric acid.

* Ann. de Chim. et de Phys., vol. xlviii. (1856) p. 153 *et seq.*

C.—Abstract of the Records of Growth of the Plants.

I.—Plants grown in 1857*.

The following list indicates the original arrangement of the experiments in 1857; but, as the records will show, beans sown and resown under shades Nos. 5, 10, and 11 died before they had attained any material amount of growth; and hence the products in these cases were not submitted to analysis.

Series 1. With no other combined nitrogen than that contained in the seed:—

1. Wheat; in prepared soil.
2. Barley; in prepared soil.
3. Barley; in prepared pumice.
4. Beans; in prepared soil.
5. Beans; in prepared pumice.

Series 2. With a supply of known quantities of combined nitrogen beyond that contained in the seed:—

6. Wheat; in prepared soil.
7. Wheat; in prepared pumice.
8. Barley; in prepared soil.
9. Barley; in prepared pumice.
10. Beans; in prepared soil.
11. Beans; in prepared pumice.

And also—

12. Wheat, Barley, and Beans, together; in rich garden soil.

Records of Sowing, and Early Stages of Growth, of all the Plants collectively.

May 12.—The weighed seeds of wheat (Nos. 1, 6, & 7), of barley (Nos. 2, 3, 8, & 9), and of beans (Nos. 4, 5, 10, & 11) were respectively put into small bottles, a few septems of pure distilled water added to soak them, and then corked up.

May 16.—The wheats (Nos. 1, 6, & 7), and the beans (Nos. 4, 5, 10, & 11), were sown, and the pots removed to their places on the stand, and covered with the shades; seeds all swelled; some sprouting.

May 20.—The barleys (Nos. 2, 3, 8, & 9), freshly weighed seeds (the soaked ones being abandoned), were set, and the pots removed to their position under the shades.

May 27.—Nearly all show shoots above the surface, all of which look green and healthy.

June 2.—Wheat and barley plants two or three leaves each, healthy, but pale green. No. 4 beans (soil) healthy and vigorous. No. 5 beans (pumice) one plant up, with three leaves speckled with black spots; the other plant blackened and apparently dead. Beans No. 10 (soil) and No. 11 (pumice) slightly speckled with black spots.

June 3.—Commenced the daily passage of washed air over the plants, in quantity

* The figures (Plate XV.) of the plants grown in 1857 are reduced from drawings taken, for the most part, about the middle of August.

equal to about $2\frac{1}{2}$ times the volume of the shade. Carbonic acid also daily supplied, in amount as described at pp. 480, 481.

June 6.—Graminaceous plants (Nos. 1, 2, 3, 6, 7, 8, & 9) all healthy, though with a tendency to turn yellow at the tips of the leaves. Of the Leguminous plants, Nos. 5, 10, and 11 give indications of dying.

June 8.—Some of the wheat and barley plants turning yellow. Beans Nos. 5, 10, and 11 obviously dying; probably injured by the causticity of the ash added to the soil, as No. 4 beans, the seeds and roots of which happen to be washed when water is supplied, are healthy and vigorous.

Records for each Experiment given separately.

No. 1.—*Wheat* (1857); *six seeds; prepared soil; without nitrogenous manure.*

(See Plate XV. fig. 1.)

June 9.—Five plants up; one quite small, the others 2 to 4 inches high, with two leaves developed and a third appearing; yellowish at the tips of some of the leaves.

June 15.—Five healthy plants, each with three fully developed leaves; tips of the lower leaves slightly yellow.

June 24.—Plants 5 inches high; lower leaves dead and dry, upper pale green; with some of the tips yellow, but general appearance of the upper leaves healthy.

July 4.—Plants 6 to 7 inches high; 5 leaves on each; upper ones pale green, lower ones yellow.

[Note.—Drops of water condense rapidly on the tips of the leaves of all the Cereals, but not of the Leguminous plants; they also form and run down the inner surface of all the shades, casting focal rays apparently injurious to the plants when not shaded from direct sunlight.]

July 11.—Same number of leaves; very little further growth; lower leaves more dried up.

July 22.—Very little improvement.

July 29.—Very little growth, though upper leaves continue green; but little tendency to form stem.

[Note.—Shade opened a few seconds to substitute a tube for one accidentally broken.]

August 10.—Green colour maintained, but no apparent increase in size.

August 24.—Five plants, 6 to 9 inches high, with eight or nine leaves each, all dried up but the two upper ones, which are green and healthy, one expanded, the other folded in the axis of growth. The healthy appearance of the upper leaves has been maintained several weeks, with otherwise almost total cessation of growth.

October 3.—*Plants taken up:*—

The plants have been almost stationary since the last report; termination of the ascending axis keeps green; no indication of heading. (See Plate XV. fig. 1.)

Soil moist, soft, and spongy.

Roots not distributed generally throughout the soil; a few isolated ramifications

extended to the lower part of the pot ; but the great mass remained near the base of the stem. Total quantity of root very small compared with that of wheat No. 6 manured with ammonia-salts. For general character of root-development, see Plate XV. fig. 15.

For method of further treatment see pp. 543, 544.

No. 2.—*Barley* (1857) ; *six seeds ; prepared soil ; without nitrogenous manure.*
(See Plate XV. fig. 2.)

June 9.—Six plants ; 2 to 3 inches high, with two fully developed leaves ; tips of some of the leaves slightly yellow.

June 15.—Three plants with three leaves, and three with two leaves each ; tips of lower leaves slightly yellow, but general appearance healthy.

June 24.—Plants 4 to 6 inches high, with three or four leaves each ; much the same condition as wheat No. 1 at this date.

July 4.—6 to 7 inches high, with four or five leaves ; paler than wheat No. 1 ; looking sickly. Drops of water on tips of leaves and inner surface of shade : see Note thereon to wheat No. 1, same date.

July 11.—Lower leaves drying up ; upper ones growing a little, apparently at expense of the lower. Stems of these and the other barley plants reddish, and have been so since the formation of true stems with nodes. The barleys form stem more readily than the wheats, which are more leafy.

July 22.—Not much improvement.

July 29.—Only two small leaves at the top green ; the amount green at one time does not increase ; lower leaves dry up as new ones form.

August 10.—Very little change, except that one stem shows slight indications of heading.

August 24.—*Plants taken up* :—

Six plants, 5 to 17 inches high, with six to nine leaves on each plant. Two indicate slight tendency to heading, the sheath being swollen ; but growth obviously ceased, the two upper leaves having at last lost colour and dried up. On opening, one head showed a rachis 2 inches long. The plant was very dry, so no fresh weight taken.

Prepared and analysed as described at pp. 543, 544.

No. 3.—*Barley* (1857) ; *six seeds ; prepared pumice ; without nitrogenous manure.*
(See Plate XV. fig. 3.)

June 9.—Six plants, $2\frac{1}{2}$ to 4 inches high ; more developed, but more slender than the barleys in soil (Nos. 2 & 8). Leaves turning yellow at the tips.

June 15.—Six plants, 6 inches high, each with three fully developed leaves ; tips of lower leaves dried up ; middle leaves have yellow tips ; upper ones pale green but healthy. Plants appear to have almost done growing.

June 24.—Height about the same ; three or four leaves each plant ; lowest dried up, next drying, and upper ones green.

July 4.—Plants 6 to 7 inches high ; five or six leaves each ; upper leaves only pale green.

[Drops of water collect as described in reference to No. 1 Wheat at this date.]

July 11.—Plants 6 to 8 inches high ; five or six leaves each ; upper ones green and growing a little as the lower ones dry up ; general aspect stationary.

July 22.—Very little growth.

July 29.—Plants 8 to 10 inches high, very slender, like mere threads ; all lower leaves dried up ; upper ones 1 to 2 inches long and pale yellow. The six plants show twenty nodes. Slight tendency to form very small heads.

August 10.—Plants quite dried up.

August 25.—*Plants taken up* :—

Six very slender plants, mere filaments, 8 to 20 inches long ; with four to six nodes, and six to eight leaves each. Stems zigzag at the nodes ; leaves dried up and brown. The top sheath of five of the plants indicates an excessively small head with zigzag rachis, at the upper part of which is a well-defined husk but no seed ; the lower parts have beards and small rudimentary husks.

Preparation and analysis as described at pp. 543, 544.

No. 4.—*Beans* (1857) ; *two seeds ; prepared soil ; without nitrogenous manure.*

June 9.—Two plants up ; one 6 inches high, four leaves with two leaflets each and two large stipules ; the other smaller ; both healthy and vigorous.

June 15.—One plant $7\frac{1}{2}$ inches high, with five leaves, each with two or three leaflets and two stipules ; the other $3\frac{1}{2}$ inches high, with four leaves and corresponding stipules. Tips of some of the lower leaves slightly speckled, but the upper ones green, and both plants healthy and vigorous.

June 24.—One plant 15 inches high, with seven leaves, each with two or three leaflets and two stipules ; lower leaves yellow, with dark specks at the edge, upper leaves and stem light green ; the other plant 9 inches high, four or five leaves with two to three leaflets, &c., each ; lower leaves as on the other plant, but upper ones greener. Plants appear to have nearly done growing.

July 4.—One plant 19 inches high ; five leaves fallen off within two days, three upper ones remain, these green, appear to live on nutriment drawn from the lower ones. The other plant 12 inches high, seven leaves, and a small sprout just at the surface of the soil ; lower leaves dead, upper ones nearly done growing.

July 5.—*Plants taken up** :—

Preparation and analysis as described at pp. 543, 544.

* After removal of the beans, a barley plant from the field was potted with its own soil which was comparatively dry, and placed under the shade without being watered, in order to see whether water was given off and condensed within the glass as freely as in the case of the experimental plants. It was so; and hence it was concluded that the experimental soils were not too wet.

No. 5.—*Beans* (1857); *two seeds; prepared pumice; without nitrogenous manure.*

June 9.—One plant $1\frac{1}{2}$ inch high, blackened, and dying; the other smaller and already dead.

As will be seen by the records (p. 557), Beans Nos. 10 & 11 showed equally unhealthy growth; all were therefore removed and re-planted. It was obvious that the failure was too early to be due to want of available nitrogen; especially, as No. 4 Beans with a similar amount of nitrogen lived. The result was considered to be due to the causticity of the ash, as beans set in ash-free soil and pumice flourished much longer, and in the case of No. 4 the seeds happened to be so placed as to be washed when water was applied.

It was found on examination that all showed signs of recommencement of growth; new roots and stems were forming. The seeds, &c. were removed; a little sulphuric acid added to the soil (or pumice) to neutralize the ash, and it was then ignited as originally, put into fresh red-hot pots, and cooled and moistened over sulphuric acid. Before putting in fresh seeds, holes were made for them in the soil, and water poured in to remove soluble matter from the neighbourhood of the young rootlets. The experiments were then continued as before.

Report of No. 5 Beans continued.

June 24.—One plant just up.

July 1.—An accident occurred to this experiment. A fresh pot of soil, prepared precisely as above, was planted with beans that had been set in small glass tubes ready for any contingency, and the experiment continued.

July 4.—One plant, leaves just opening.

July 11.—Still only one plant up, and it looks very unhealthy.

July 22.—One plant, obviously dying.

July 29.—Dead.

No. 6.—*Wheat* (1857); *three seeds; prepared soil; with nitrogenous manure.*

(See Plate XV. fig. 7.)

June 9.—Two plants up; one $2\frac{1}{2}$, the other $4\frac{1}{2}$ inches high; three leaves each. Tips of leaves slightly yellower than those of Wheat No. 1.

June 10.—A pipette-ful of the solution of sulphate of ammonia (=·00578 gramme N.) added to the soil.

June 15.—Two plants; green and vigorous; marked improvement since the addition of ammonia-salt; the leaves wider and of a deeper green. Three leaves each plant.

June 24.—Two plants; 7 inches high; four or five leaves each; lower ones dried up, upper ones deeper green than Wheats No. 1.

July 4.—Two plants; 9 inches high; six leaves each; lower ones yellow, upper ones broad, long, and of a healthy deep green; but the vigour due to the first addition of

ammonia appears to have ceased. Second pipette-ful of ammonia-solution (same quantity) added.

July 11.—Two plants, 10 inches high ; seven leaves each ; upper ones deep green, broad, and vigorous. Third pipette-ful of the ammonia-solution added.

July 22.—Growth vigorous ; shooting out at the base of the stems. Fourth pipette-ful of the ammonia-solution added.

July 29.—Much greater tendency to form leaf than stem. One plant with four, and the other with two subdivisions. 12 to 16 inches high, the height greatly due to the length of the leaves. Not a single node clear of the sheath of the one below it ; thus essentially different from the barleys, which have great tendency to form nodes and stem. Fifth pipette-ful of the ammonia-solution added.

August 10.—Green and flourishing.

August 24.—Plants 17 to 20 inches high ; ten to twelve leaves on each ; upper ones long, broad, and green ; lower ones dried up. But little tendency to form stem ; leaves larger than on plants in the field ; some 12 inches long and $\frac{1}{2}$ inch wide ; no nodes clear ; the leaves spring out so close together as to appear almost opposite. Five stems from the two seeds.

October 2.—*Plants taken up* :—

One seed has given three strong and one small stem ; another one stem ; the third did not grow. Leaves very numerous and close together, giving several thicknesses of sheath around the stem, and hiding all the nodes ; lower leaves dried up ; upper leaves and central axis of growth green. Condition nearly stationary for the last two or three weeks. Average height of plants about 18 inches.

Soil quite moist throughout ; also soft, and spongy, rather more so than the pumice soils ; a little water remained in the plate below the pot.

Roots much, but very irregularly distributed—a large bunch around the base of the stem ; small, long, isolated roots extended to the bottom and up the sides of the pot ; quite a mass of ramified roots over the bottom, and somewhat up the sides of the pot ; and a greater mass in the dish under the pot, forming a circular web the size of the bottom of the pot. A crack in the bottom of the pot was penetrated with roots throughout, showing, perhaps, that more openings than the one hole at the bottom might be advantageous. For representation of the root-development, see Plate XV. fig. 14.

Preparation and analysis as described at pp. 543, 544.

No. 7.—*Wheat* (1857) ; *three seeds ; prepared pumice ; with nitrogenous manure.*

June 9.—Three plants up, 3 to 4 inches high ; each with three leaves completely formed, of which the tips are slightly yellower than those of Nos. 1 and 6, but no appearance of diseased condition in any of the wheats.

June 10.—A pipette-ful of the ammonia-solution (=·00578 gramme N.) added to the soil.

June 15.—Plants 5 to 6 inches high, with four leaves each ; the tips of the lower

ones yellow; the newer and upper leaves green, healthy, and vigorous; marked improvement since adding the ammonia-solution on June 10, the effect of which was manifest within two days after the addition.

June 24.—Plants 5 to 7 inches high, with four leaves each; lower leaves dried up, but upper ones green and vigorous; obviously improving; forming stem with nodes.

July 4.—Plants 7 to 8 inches high, with five to seven leaves each; the newer ones broad, well developed, and of a deep green colour; upon the whole vigorous. Second pipette-ful of the ammonia-solution added.

[Drops of water accumulate as described in reference to No. 1 of this date.]

July 11.—Plants 8 to 9 inches high, with six or seven leaves each; lower ones pale yellow, upper ones green and vigorous. One of the stems sending out a shoot at its base. Third pipette-ful of ammonia-solution added.

July 22.—Growing very well; tillering very much. Fourth pipette-ful of the ammonia-solution added.

July 29.—Plants 12 to 16 inches high; one with six shoots 4 to 8 inches long; one with one shoot 3 inches long; and the other with two shoots just forming; shoots, and upper leaves, green. The ammonia seems to induce multiplication of shoots instead of upward growth; no nodes clear of the sheath. Fifth pipette-ful of the ammonia-solution added.

August 10.—Green and flourishing.

August 24.—Very similar to Wheat No. 6 at this date.

September 20.—*Plants taken up*:—

The lower leaves begin to lose colour considerably, no increase of growth apparent for some days, nor any tendency to form seed; hence, the season being far advanced, the plants taken up.

Great development of root; the plate under the pot covered with a dense network ramified from a few fibres extended to the bottom of the pot; a similar network at the bottom and partially up the sides within the pot; comparatively little in the centre of the soil.

Preparation and analysis as described at pp. 543, 544.

No. 8.—*Barley* (1857); *four seeds; prepared soil; with nitrogenous manure.*
(See Plate XV. fig. 8.)

June 9.—Three plants up; two $1\frac{1}{2}$ inch and one $3\frac{1}{2}$ inches high; colour pale.

June 10.—A pipette-ful of ammonia-solution (=·00578 gramme N.) added to the soil.

June 15.—Three plants; about $4\frac{1}{2}$ inches high; each with two leaves and another forming. Improved by the ammonia added June 10, but not so much as the Wheat No. 6.

June 19–20.—During the night the shade was cracked, from the bottom in the quicksilver, 9 inches upwards. The pot with its contents was removed and put under a shade over sulphuric acid. After four days it was returned to its place, and covered with the

shade of Experiment No. 12 (with plants in garden soil), the latter being replaced by the damaged shade after the crack had been mended with strips of bladder cemented with albumen and lime-water. All the circumstances of this accident were carefully considered, and it was concluded that no appreciable error could arise from it.

June 24.—Three plants, 3 to 5 inches high; three or four leaves each; lower ones dried up, upper ones pale green; plants slender, but improved since the addition of the ammonia-solution.

July 4.—Plants 6 to 7 inches high; five leaves each; the most delicate and slender of the plants that have had ammonia-solution; upper leaves darker green than those without ammonia; lower leaves yellow. Second pipette-ful of ammonia-solution added.

[The same remarks apply here, as were made to No. 1 at this date, in reference to condensation of drops of water.]

July 11.—Plants 7 to 9 inches high; six or seven leaves each; stem reddish; upper leaves healthy and deep green. Third pipette-ful of ammonia-solution added.

July 22.—Growing vigorously. Fourth pipette-ful of ammonia-solution added.

July 29.—Four plants, 16 to 20 inches high. Since the last two additions of ammonia-solution, two of the plants have sent out at the base two new shoots, 6 to 8 inches high; one, two new shoots 2 to 4 inches high; and the other, one shoot. All these shoots are deep green and growing vigorously. A great tendency to develop new foliage; and though some of the stems were just beginning to swell, indicative of heading, and one showed a beard, yet this growth was arrested, and the energies of the plant directed to the new growths at the base. In all, seventeen nodes clear of the sheaths. Fifth pipette-ful of the ammonia-solution added.

August 10.—Since the last three additions of ammonia the old stems ceased to develop, but some of the new ones are on the point of heading.

August 24.—Eight plants from the three seeds. One seed has given one plant 24 inches high, with seven nodes clear, and nine leaves, of which the seven lower ones are dried up; the plant terminated by a well-formed head. Another seed has four stems, 16 to 20 inches high; one dried up just as it was heading; the three others green and healthy, and two just commencing to head; each stem four to six nodes, and six to ten leaves. The third seed has three stems 12 to 24 inches high, each with three to five nodes and five to ten leaves; one stem dried up.

October 8.—*Plants taken up*:—

Eight stems from three seeds, as under:—

(*a*) Seed with one stem; 18 to 20 inches high; seven nodes. This was the first plant that headed; all ripe and dry; six glumes, containing only rudimentary or undeveloped seeds.

(*b*) Seed with three stems. One 17 inches high; head ripe, and rather decaying. Another 25 inches high; grown several inches, and formed head, since August 24; head green, with five soft milky unripe grains. The third stem green at top, and upper sheath swollen with the head.

(*c*) Seed with four stems :—(1) 22 to 23 inches high, with green head and six unripe grains; leaves dry and ripe; (2) stem 15 inches high, dried up, head-sheath formed; (3) 19 inches high; yellowish-green head, with nine glumes, and undeveloped seeds; (4) about 15 inches high; rather green, sheath swollen, and beard appearing.

During the last three weeks some heads came out more, and indications of others developed; otherwise not much change. From the low temperature and lateness of the season, it was thought the plants would not mature further.

Preparation and analysis as described at pp. 543, 544.

No. 9.—*Barley* (1857); *four seeds; prepared pumice; with nitrogenous manure.*
(See Plate XV. fig. 9.)

June 9.—Four plants; one quite small; the others 3 to 4 inches high. These more grown than the Barley plants Nos. 2, 3 & 8; but the leaves, particularly the lower ones, yellower at the ends.

June 10.—A pipette-ful of ammonia-solution (=·00578 gramme N.) added to the soil.

June 15.—Four plants; 5 to 6 inches high; four leaves each; lower ones losing vitality. Lower leaves were too far gone, but a most marked improvement in the upper ones since the ammonia-salt was added; it was manifest in two to three days after the addition.

June 24.—Four plants; height 6 to 8 inches; improved very much by the addition of the ammonia-solution.

July 4.—Plants 8 to 13 inches high; six or seven leaves each; stems very slender, but show well-formed nodes. Second pipette-ful of ammonia-solution added.

[Drops of water accumulate as described in reference to No. 1 of this date.]

July 11.—Plants 9 to 14 inches high; seven or eight leaves each; upper ones deep green; lower ones yellow; stems red. Third pipette-ful of ammonia-solution added.

July 22.—Growing very well; showing indications of heading. Fourth pipette-ful of ammonia-solution added.

July 29.—The four plants all out in head; about 30 inches high; each stem six nodes; two of the plants have shoots 5 inches high. The ammonia seems to tend more to new growth than to the development of the old.

August 10.—Heads well developed.

August 24.—The plants appear to be ripening; heads turning brown; but one new stem is still green and growing.

September 24.—*Plants taken up*:—

Seven plants; five 2 to 2½ feet high, one green; one 1½ foot high, green head; one 14 inches high, green. Six with heads, four ripe and two green; the shortest plant with green leaves and without head. Heads 1½ inch long; glumes all along the rachis, but only some with grains.

Roots by no means so abundant as those of Wheat with ammonia-salt; only a few fibres extended through the hole at the bottom, or to the sides of the pot.

Preparation and analysis as described at pp. 543, 544.

No. 10.—*Beans* (1857); *two seeds; prepared soil; intended to have nitrogenous manure.*

June 9.—Only one plant up; 2 inches high; turning black and obviously dying.

For particulars of taking up, setting fresh seeds and recommencement of the experiment, see remarks made on June 9 to Bean No. 5, p. 552.

June 15.—Not yet up.

June 24.—Two plants just appearing.

July 4.—Two plants well up and growing; leaves just opening.

July 11.—Two plants; 6 to 8 inches high; leaves deep green.

July 22.—Green, healthy, and vigorous.

July 29.—Nearly as at last date, but somewhat declining.

August 10.—Obviously dying.

August 24.—Dead.

The season too far advanced to repeat this experiment.

No. 11.—*Beans* (1857); *two seeds; prepared pumice; intended to have nitrogenous manure.*

June 9.—One up; slender; black spots on the leaves; obviously unhealthy. Taken up, and the experiment recommenced; for particulars of resetting, &c., see remarks to Bean No. 5 of this date, p. 552.

June 15.—Not yet up.

June 24.—Two plants just up.

July 11.—Apparently not going to grow.

July 22.—Dead; the season too far advanced to repeat this experiment.

No. 12.—*Wheat, Barley, and Beans* (1857); *Wheat and Barley three seeds each, Beans two seeds; in rich Garden soil.* (See Plate XV. fig. 13.)

May 18.—Seeds of wheat, barley, and beans, all sown together in a single pot of good garden soil, and placed under a shade (No. 12), to be supplied with washed air, &c., just as in the other experiments. The seeds germinated well.

May 28–29.—During the night, owing to a leakage of water from the reservoir into the vessel A (see description at p. 476 *et seq.*, and Plate XIII.), it passed over into the sulphuric acid and carbonate of soda wash-bottles, and the mixed liquid passed into the shade to the depth of some inches, and destroyed the experiment.

May 30.—Plants from seeds which had been set at the same date as the foregoing, were transplanted into a fresh pot of garden soil, which was placed under the shade, and the experiment recommenced. The wheat and barley plants were about 5 inches, and the beans about 4 inches high.

June 15.—Healthy, and growing vigorously.

June 24.—Three wheats, three barleys, and two beans. Wheat 14 inches, barley

13 inches, and beans 11 inches high. Wheat and barley much branched at the base, giving fourteen stems from the six seeds; all a deep green colour. Beans deep green, and growing well, excepting that one has a few black specks on the lower leaves. So much growth that the plants are considerably crowded in the shade.

July 4.—Much crowded. Graminaceæ 20 inches, Leguminosæ 15 inches high. The former growing as well as in the open air. The latter appear to suffer from crowding; their lower leaves dying.

July 12.—The Graminaceæ growing very healthily; Leguminosæ apparently not so.

July 22.—The Graminaceæ growing vigorously; Leguminosæ revived, and also growing vigorously at the top. During the last few days they have been protected from the direct sun by a sheet of paper tied round the shade.

July 29.—Four barleys in head; wheat not so advanced, but nearly as high; the beans had again suffered, but one is recovering. Too much crowded.

August 10.—About as at last date.

August 24.—About as at last date; barley slowly ripening.

The object of the experiment being attained, which was to determine whether the conditions of atmosphere were suited to healthy growth, provided the soil supplied sufficient nutriment, no further records of growth were made.

II. Plants grown in 1858*.

As in the experiments of 1857, so in those of 1858, the plants grown may be divided into two Series, as under :—

Series 1. With no other combined Nitrogen than that contained in the seed sown.

Series 2. With a supply of known quantities of combined Nitrogen beyond that contained in the seed.

The notes of growth of the plants grown without any extraneous supply of combined nitrogen are given first, and then those of the plants grown with such supply. As before, in several experiments instituted with Leguminous plants they died before attaining a sufficient amount of growth to render it of any use to analyse the products. The records of their progress, such as it was, are, nevertheless, shortly given.

No. 1.—*Wheat* (1858); *eight seeds; prepared soil; without nitrogenous manure.*

(See Plate XV. fig. 4.)

April 27.—Seeds set, and the pot placed under a shade over sulphuric acid.

May 7.—All the plants up; the pot removed to its shade on the stand.

May 20.—Eight plants; all of a healthy green colour; seven 4 inches high, one just above the soil.

* The figures (Plate XV.) of the plants grown in 1858 are reduced from drawings taken, in most cases, not many days before the plants were taken up.

May 22.—A pipette-ful of the sulphuric-acid solution added.

May 29.—Eight plants, 4 to 6 inches high; each with four leaves, the two lower yellow, the two upper green and healthy. A drop of water appears on the tip of the upper leaves in the morning, but it disappears before midday, as the air is passed through the shade. A pipette-ful of the phosphate-solution added.

June 7.—A pipette-ful of the phosphate-solution, and a pipette-ful of the sulphuric-acid solution added.

June 19.—Plants 5 to 7 inches high; two lowest leaves on each dried up; upper ones yellowish green.

June 26.—Eight plants, 6 to 7 inches high; six leaves each, three lower ones dried up, next two pale green, only upper central one green and healthy. Apparently at limit of growth without more combined nitrogen; very much as last year without nitrogenous manure.

July 3.—A pipette-ful of the phosphate-solution, and a pipette-ful of the sulphuric-acid solution added.

July 14.—Plants 6 to 8 inches high, with six or seven leaves each; only the two upper ones yellowish green; apparent stagnation of growth.

July 29.—Much as last; two upper leaves seem to sustain life at the expense of the rest.

August 17.—After long inactivity several plants show tendency to grow in *stem*. In this, somewhat more like the barley than wheat of last year. Some disposition to heading.

September 7.—Still developing stem; nodes and internodes distinctly marked. Plant (*a*) 13 inches high, ten leaves, three nodes bare, slightly swelled at top as if heading; new stem-leaves, only 2 to 3 inches long. Plants (*b* and *c*) $9\frac{1}{2}$ inches high, nine leaves, two or three bare nodes; slight indication of heading. Plants (*d*, *e*, and *f*) $7\frac{1}{2}$ inches high, two bare nodes; stems shorter, leaves eight or nine, a little longer than above. Plant (*g*) two branches; the first short, and dried up; a new one formed from its base, green, but only $4\frac{1}{2}$ inches high, with four green leaves. Plant (*h*), dried up stem with three long leaves; but a new green shoot with two leaves, though little growth. General remark:—all lower and first-formed leaves dried up, the next yellowish, and only the two upper ones green. Drops of water collect at the tips, and axils, of the green leaves. The later growth obviously at the expense of the earlier.

October 5.—Little change, except riper. Plant (*a*) 14 inches high, eleven leaves, nearly all dried up, four bare nodes, a head with indications of seeding: (*b*) $10\frac{1}{2}$ inches high, eleven leaves, all ripe but the uppermost, three bare nodes, and indication of heading: (*c*) $9\frac{1}{2}$ inches high, nine leaves, three nodes: (*d* and *e*) $8\frac{1}{2}$ inches high, eleven leaves each: (*f* and *g*) 4 to 7 inches high, dead stems with eight to ten leaves each, but green shoots at the base: (*h*) 7 inches high and seven leaves, dead ripe.

October 24.—Weather much warmer again lately, and slight renewal of growth; drops of water again appear on the green top leaves. The chief growth is further deve-

lopment of the rudimentary head ; a definite rachis formed, with joints and rudimentary husks, but no indication of seed.

October 25.—*Plants taken up* :—

Soil quite wet, loose, and open, to the bottom ; roots pass through the pot at nearly all the bottom holes, and at some of the side ones ; long roots distributed among the flints ; very few roots come to the sides of the pot (see Plate XV. fig. 17).

Plant 1. Dead ripe, 7 inches high, seven long leaves, one dead shoot ; roots long, apparently going to the bottom, very little distributed.

Plant 2. Seven inches high ; two stems ; one with six leaves, dead ripe ; the other with three leaves, one still slightly green ; no nodes visible ; each, a moderate amount of root.

Plant 3. Eight inches high ; ten leaves, lower long and dead, two upper green ; no nodes visible. Many roots at the base, some extending downwards. Roots of this and all the plants have short forked branches, $\frac{1}{4}$ to $\frac{1}{2}$ inch long, blunt and thick, and generally forked at the end ; strikingly different from the roots among the loose flints at the bottom, and those under the pot.

Plant 4. Height $10\frac{1}{2}$ inches ; thirteen leaves ; six visible nodes ; slight swelling at the head. Fewer roots branched and distributed in the soil near the base of the stem ; most go to the bottom, or even under the pot, thus taking nutriment from the water in the dish rather than from the soil ;—perhaps associated with this the superior growth over plants 1, 2, and 3.

Plant 5. Very similar to No. 4.

Plant 6. Very similar to Nos. 4 and 5 ; but the head rather more developed, and visible through the transparent sheath, and the roots with rather more the character of pot or *soil* roots.

Plant 7. Eleven inches high ; twelve leaves ; five nodes visible ; head with chaff without grain, and beard $\frac{3}{4}$ inch long ; rachis 1 inch long. Roots but little branched, going down and developed more at the bottom and in the dish than in the soil.

Plant 8. The largest and most developed plant. Fourteen inches high ; twelve leaves ; lower ones long and crowded, upper ones shorter and further apart (as in all) ; four nodes ; head with rachis $1\frac{1}{4}$ inch long, with glumes and pales. Roots very similar to No. 7, forming under the pot a thick matted mass, running round the dish, some of which, when untangled, are 3 to 4 feet long ; white, transparent, and with many small thread-like branches ; the whole somewhat resembling a mass of white thread.

Preparation and analysis as described at pp. 543, 544.

No. 2.—*Barley* (1858) ; *eight seeds ; prepared soil ; without nitrogenous manure.* (See Plate XV. fig. 5.)

April 27.—Seeds set, and the pot placed under a shade over sulphuric acid.

May 7.—Pot removed to its shade on the stand.

May 20.—Five plants 4 inches high, and one 1 inch. Were at first quite green and healthy, but the last few days turning yellowish green.

May 22.—A pipette-ful of the sulphuric-acid solution added.

May 29.—Five plants 4 to 5 inches high, with three or four leaves each ; lower ones yellow and dried up ; upper pale yellowish green. A sixth plant, smaller. A pipette-ful of the phosphate-solution added.

June 7.—A pipette-ful of the phosphate-solution, and a pipette-ful of the sulphuric-acid solution added.

June 19.—One plant dead; two about 4 inches high with shoots at the base ; other two about 8 inches high.

June 26.—Plant (*a*) dead ; cause not obvious. Plant (*b*) 10 inches high ; as last year, forming stem well. Plant (*c*) 8 inches high. Plant (*d*) a main stem which is dead, and a new shoot which is green (each 3 to 4 inches high). Plant (*e*) a good deal like (*d*).

July 3.—A pipette-ful of the phosphate-solution, and a pipette-ful of the sulphuric-acid solution added.

July 14.—Plant (*b*) 9 to 10 inches high ; six dried up and two green leaves ; swelling apparently for heading. Plant (*c*) about 7 inches high ; seven dried up and two green leaves. Plant (*d*) two stems 4 to 6 inches high ; six dried up and two green leaves. Plant (*e*) two stems 4 to 6 inches high, with five dead and two green leaves.

The upper leaves quite short (1–1½ inch long), and apparently live at the expense of the lower.

July 29.—Plant (*a*) dead ; six leaves, becoming brown-yellow ; a black mildew has attacked the leaves and stem ; and a white gossamer-like fungus has attached itself in places to the stem and leaves. Leaves 3¼ to 4 inches long ; the upper thread-like and drooping. Plant (*b*) the most flourishing ; 14 inches high ; but very spindly ; six nodes, which, with portions of the adjoining culm, especially the upper part, are dark purplish ; eight leaves ; lower ones yellow, and the lowest two, which are in contact with plant (*a*), affected with the mildew ; all but the uppermost leaf 2 to 2½ inches long; the upper one 1½ inch long, pale green, and quite erect, apparently the last effort of the plant, no new leaves forming. Plant (*c*), divided just beneath the soil into three shoots ; two apparently suckers from the other, each 3 inches high, and dead. The main plant 6 inches high ; has seven leaves ; the four lower dead, and the three upper, making up half the plant, pale green ; the uppermost only ½ an inch long, in the fold of the second. Only one node visible ; the culm, where seen, is purplish. The white fungus occurs, but no mildew. Plant (*d*) much like the main plant (*c*) ; evidence of early effort to put out shoots at the base. Twelve leaves ; ten lower ones dead ; two upper ones living ; all 2 to 2½ inches long. Plant (*e*) the second in size. Eleven inches high ; ten leaves ; eight lower ones dead, two upper ones living ; all erect but the lowest two ; each 2 to 3 inches long.

August 18.—*Plants taken up* :—

Evidently done growing ; four stems swelled for head ; all leaves except the uppermost dried up. Roots not much distributed ; general characters much like those of barley without nitrogenous manure last year (1857). Soil moist, loose, and open.

Preparation and analysis as described at pp. 543, 544.

No. 3.—*Oats* (1858); *eight seeds; prepared soil; without nitrogenous manure.*
(See Plate XV. fig. 6.)

April 27.—Seeds set, and the pot placed under a shade over sulphuric acid.

May 7.—The pot removed to its shade on the stand.

May 22.—A pipette-ful of the sulphuric-acid solution added.

May 29.—Eight plants, 4 to 6 inches high; four or five leaves each; lower ones yellow, upper ones green and growing. These Oats growing rather better than either No. 1 Wheat, or No. 2 Barley. A pipette-ful of the phosphate-solution added.

June 7.—A pipette-ful of the phosphate-solution, and a pipette-ful of the sulphuric-acid solution added.

June 19.—Eight plants, 6 to 9 inches high; five or six leaves each, lower yellow and dead, upper green. Tips of some of the leaves injured by action of direct sun-rays.

[General note.—White paper had been tied over all the shades to screen from the direct rays of the sun; but in this case not quite high enough.]

June 26.—Eight plants; five 10 to 11 inches high, and in head; three 8 to 9 inches high; no appearance of heading, and two of them a green shoot at the base. Six or seven leaves on each plant. The rachis of the seeding plants long and crooked, with one or two seeds at top, without signs of seed below. All the plants apparently at termination of growth; remain only to see how far they will ripen.

July 3.—A pipette-ful of the phosphate-solution, and a pipette-ful of the sulphuric-acid solution added.

July 13.—*Plants taken up*:—

Eight plants, quite dead ripe for some days, having had a hot sun.

Plant (1) $13\frac{1}{2}$ inches high; five leaves; rachis $1\frac{1}{2}$ inch long, with one seed. Plant (2) $11\frac{1}{2}$ inches high; five leaves; rachis $1\frac{1}{2}$ inch. Plant (3) 12 inches high; five leaves; rachis $1\frac{1}{2}$ inch long, with two seeds. Plant (4) $12\frac{1}{2}$ inches high; with shoot appearing at base; rachis $1\frac{1}{2}$ inch long, with two seeds. Plant (5) $11\frac{1}{2}$ inches high; five leaves; with shoot appearing at base. Plant (6) 9 inches high; five leaves; and shoot at the base, 4 inches long. Plant (7) 10 inches high; five leaves; and shoot at the base, 4 inches long. Plant (8) $10\frac{1}{2}$ inches high; five leaves; rachis $1\frac{1}{2}$ inch long, two seeds. Roots only extended about 2 inches deep in the pot. Soil wet and soft; the lower part firm, but not hard.

Preparation and analysis as described at pp. 543, 544.

No. 4.—*Beans* (1858); *three seeds; prepared soil; without nitrogenous manure.*

April 27.—Seeds set, and the pot placed under a shade over sulphuric acid.

May 20.—Pot removed to its shade on the stand. Three plants up, $2\frac{1}{2}$ inches high; three leaves on each; dark green and healthy.

May 22.—A pipette-ful of the sulphuric-acid solution added.

May 29.—Plants 3 to 4 inches high; one looks to be dying; the others have specks on their leaves. A pipette-ful of the phosphate-solution added.

June 7.—A pipette-ful of the phosphate-solution, and a pipette-ful of the sulphuric-acid solution added to the soil.

June 19.—One plant dead ; another looking unhealthy ; the third 4 to 5 inches high, with five leaves, growing pretty well.

June 26.—Two plants dead ; the other growing, 7 to 8 inches high, with nine leaves, each with two stipules.

July 3.—A pipette-ful of the phosphate-solution, and a pipette-ful of the sulphuric-acid solution added.

July 14.—The third or only surviving plant has ten leaves, but looks unhealthy.

July 29.—The two dead plants fallen, moulded, and dried up. The other blackened and mouldy at the base of the stem, and thence to the top yellow ; three top leaves partly yellow, but the remainder black.

August 17.—All three entirely dead. Pot removed, but products not analysed, as there had not been sufficient healthy growth. It is difficult to account for this failure ; but it is possibly due to the very hot weather.

No. 5.—*Beans* (1858) ; *three seeds ; prepared soil without* (*but intended to have*) *nitrogenous manure.*

June 11.—Seeds set in prepared soil, with ash that had been neutralized with sulphuric acid, and gently re-ignited ; and the pot placed over sulphuric acid and covered with a glass shade.

June 21.—The pot removed to its place on the stand.

June 26.—Three plants up ; green and healthy ; four leaves, each with two leaflets, and two stipules. Plants delicate, but healthy green colour ; one shows air-roots.

July 3.—A pipette-ful of the phosphate-solution, and a pipette ful of the sulphuric-acid solution added.

July 14.—Three plants, healthy and vigorous ; 8 to 12 inches high ; eight leaves on each ; a few black specks on some of the leaves, otherwise healthy. The weather has been comparatively cool since planting till now ; but now hotter with bright sun. A few air-roots at the base of the stems.

July 29.—Three plants ; 8, 8½, and 12 inches high. Plant (*a*) lost all its leaves, except rudimentary ones at the top. A shoot 2 inches long with four small leaves about an inch from the base, more growing than the parent plant ; another shoot appearing about an inch above. Plant (*b*) very unhealthy ; lost all leaves but six small and partly black ones at the top ; a vigorous shoot 5 inches long, springing an inch from the base, seems to exhaust its strength ; another small shoot 1 inch long, about 2 inches higher up. Plant (*c*), most of the leaves dropped ; but several of the petioles remain, and are green ; some small withering leaves at the top ; two shoots starting near the base.

August 17.—Three plants ; the main stem of each lost nearly all the leaves. Each plant has living shoots with several leaves each near the base.

August 23.—*Plants taken up* :—

There has been scarcely perceptible growth for two or three weeks ; leaves nearly all off. Soil moist. Roots extend only a little way, and consist of a thick mat around the

base, much divided ; none reached the flint ; the upper ones dead, the lower living ; less root than last year.

Preparation and analysis as described at pp. 543, 544.

No. 6.—*Pea* (1858) ; *three seeds ; prepared soil ; without nitrogenous manure.*

June 5.—Three peas previously set died. Three more set to-day in prepared soil, with ash that had been neutralized with sulphuric acid, and gently re-ignited ; and the pot placed over sulphuric acid and covered with a glass shade.

June 7.—A pipette-ful of the phosphate-solution, and a pipette-ful of the sulphuric-acid solution added.

June 19.—Pot removed to its place on the stand.

June 26.—Three plants, 6 to 7 inches high, with four leaves each ; not growing well.

July 3.—A pipette-ful of the phosphate-solution, and a pipette-ful of the sulphuric-acid solution added.

July 14.—Three plants ; doubtful whether they will live.

July 29.—Three plants ; two 6 inches, and one 7 inches high ; apparently dead some days, yellow, and a few spots of mould.

August 24.—*Plants taken up* :—

All dead for some time past ; probably owing to the heat. Products submitted to analysis, but the results can only be of confirmatory value.

No. 7.—*Buckwheat* (1858) ; *seed*, 1 *gramme ; prepared soil ; without nitrogenous manure.*

August 20.—Seed sown, and the pot placed over sulphuric acid, and covered with a glass shade.

August 24.—Removed to its shade on the stand. Several plants up.

September 7.—Growing well.

October 5.—Sixteen plants, 2 to 4 inches high, four to six leaves each.

October 24.—Eighteen plants, 3 to 4 inches high, with four to six leaves each ; leaves $\frac{1}{3}$ to $\frac{3}{4}$ inch wide, but have begun to look yellow and curl up ; some plants dead. The plants appear to have attained their maximum growth without nitrogenous manure.

October 28.—*Plants taken up* :—

Eighteen plants with four to six leaves each, including the seminal opposite ones ; 2 to 3 inches high ; obviously done growing ; only five or six with green leaves remaining. Roots only 2 to 3 inches long, slim, delicate, and very little distributed. Soil quite loose, porous, and friable.

Preparation and analysis as described at pp. 543, 544.

No. 8 (1858).—Plants grown without Nitrogenous Manure in M. G. Ville's Case*.

M. Ville kindly forwarded porous pots, and glazed white pans to set them in, such

* The experiments conducted in M. G. Ville's cases were commenced later in the season than those with the shades, as we waited some time in the hope that M. Ville might be able to come over and superintend the arrangement himself.

as he used in his experiments; but as too many were broken in transit to use these entirely, it was decided to use pots and pans the same as for our other experiments in 1858. The soil, ash, &c., were also prepared in the same way as for the other experiments of 1858—the ash, however, being saturated with sulphuric acid and re-ignited before being used, as in a few only of the other cases; for description, see pp. 470–472.

Pot 1—Wheat; eight seeds.
Pot 2—Barley; eight seeds.
Pot 3—Oats; eight seeds.
Pot 4—Beans; three seeds.

June 11.—Wheat, Barley, and Oats; seeds set, and the pots placed over sulphuric acid, and covered with a shade.

June 12.—The three pots removed to M. Ville's Case.

June 19.—Wheat, Barley, and Oats all up, and looking green and healthy.

June 25.—Cereals all about the same size, with three leaves each; wheat quite pale, barley and oats green at the top, and lower leaves dead.

The case was opened, and the pot of three beans put in.

July 1.—Wheat pale and blanched; barley and oats, lower leaves dead, upper ones green and growing.

July 3.—All the Cereals getting quite pale; beans healthy.

July 14.—Bean, three plants up; two cotyledons and two leaves on each, healthy. Wheat, seven plants; 4 to 6 inches high; four leaves on each, upper green, lower dead. Oats, seven plants; very like the wheat; but little more disposition to form stem. Barley, eight plants; much like the oats.

August 2.—Beans, 4 inches high; lower leaves dead, and show a white mould; small stems putting out languidly. Barley, eight plants; 4 to 5 inches high; lower leaves yellow and dead; upper blades green; stems appear mouldy. Oats, seven plants about 5 inches high; lower leaves dead and mouldy; upper part of stem green, and slightly swelled as if going to head. Wheat, very much like the barley.

August 17.—Cereals appear to have nearly done growing; beans dying.

September 7.—Beans dead. Wheat green at the tops, but dead below; 4 to 6 inches high; about ten leaves each. Oats nearly dead. Barley nearly dead.

October 24.—Wheat still green at the top; oats and barley dead. Beans dead (not analysed).

November 6.—*Plants taken up.* Notes as under :—

Wheat. Seven plants; 4 to 5 inches high; no nodes visible; little tendency to form stem; lower leaves dead; top leaf green, and on some the next greenish yellow. Soil moist. Very little root.

Oats. Seven plants, 4 to 6 inches high; about six leaves each; generally four nodes visible; most forming head. Roots but little distributed; very little below $1\frac{1}{2}$ inch; fewer and more slender than those of the wheat. All the plants dead or ripe, but not decomposed; the stems firm and elastic.

Barley. Eight plants, 4 to $6\frac{1}{2}$ inches high; two to four nodes visible in all; six or

seven leaves on each ; most have a leaf-like heading stretching upwards ; lower leaves generally very long, 4 or 5 inches. Roots very little distributed ; perhaps a little deeper than the oats ; but few deeper than $1\frac{1}{2}$ to 2 inches. Soil dry, loose, and porous ; flints quite open.

Wheat, Oats, and Barley prepared and analysed as described at pp. 543, 544.

Plants grown in 1858, *with a supply of combined Nitrogen beyond that contained in the seed sown.*

No. 9.—*Wheat* (1858) ; *four seeds ; prepared soil ; with nitrogenous manure.*
(See Plate XV. fig. 10.)

April 27.—Seeds set, and the pot placed over sulphuric acid, and covered with a glass shade.

May 20.—Four plants up ; healthy, green, and growing ; about 4 inches high ; very similar to Wheats No. 1 of this date.

May 22.—A pipette-ful of the sulphate-of-ammonia solution (= 0·004 gramme N.) added to the soil.

May 29.—Four plants, from 4 to 6 inches high. Greener and fresher since the addition of the ammonia-solution ; new shoots appearing ; only the lowest leaf on each yellow. A pipette-ful of the phosphate-solution added.

June 7.—Second pipette-ful of the sulphate-of-ammonia solution added ; and a pipette-ful of the phosphate-solution.

June 19.—Four plants ; one with four shoots or stems, and two others with two stems each ; in all nine stems or plants ; 5 to 7 inches high ; leaves more healthy, green and vigorous.

June 21.—Third pipette-ful of ammonia-solution added. Plants had begun to show the want of available nitrogen.

June 26.—Fourth pipette-ful of the ammonia-solution added. As intimated May 29, the ammonia tends much to new shoots from the base. The four seeds have given—No. 1, one stem, with seven leaves : No. 2, two stems, each with six leaves : No. 3, three stems, each with five or six leaves : No. 4, four stems, with four, five, or six leaves each : in all ten stems. The two lowest leaves dead, the others green and vigorous. More vegetable matter from these four seeds, with ammonia, than from the eight (No. 1 Wheat) without it. Plants 8 to 11 inches high, and improve with each addition of ammonia.

July 1.—The shade slightly cracked at the bottom during the night, but still sufficiently air-tight for changes of temperature to affect the level of the sulphuric acid in the bulb-apparatus. Shade replaced by a small one temporarily. An immaterial amount of condensed water lost. Many roots found to be distributed through the bottom of the pot, and growing in the dish beneath it.

July 3.—Fifth pipette-ful of ammonia-solution added. [It is intended that none of the plants shall suffer so much for want of combined nitrogen, as in 1857.] Also a pipette-ful of the phosphate-solution added.

July 12.—Sixth pipette-ful of ammonia-solution added.

July 14.—Seventh pipette-ful of ammonia-solution added. Details of growth from the four seeds as follow :—

No. 1, one stem only, 8 to 10 inches high ; three upper leaves green and vigorous, four lower yellow. No. 2, three stems : (*a*) 3 to 4 inches high, two yellow and two green leaves ; (*b*) 4 to 6 inches high, two yellow and two green leaves ; (*c*) 12 to 14 inches high, three yellow and three green leaves. No. 3, three stems : (*a*) 4 to 6 inches high, two yellow and two green leaves ; (*b*) 8 to 10 inches high, two yellow and three green leaves ; (*c*) 13 to 15 inches high, two yellow and three green leaves. No. 4, four stems, 8 to 10 inches high, three yellow and three green leaves each.

The "yellow" leaves are small ones at the base, developed before ammonia was added, and are dead. The ammonia strikingly develops the new shoots, and their leaves are much larger. As last year, much more tendency to form leaf than run to stem. The height given above includes that of the erect leaves extending above the ascending axis.

July 19.—Eighth pipette-ful of ammonia-solution added.

July 29.—Ninth pipette-ful of ammonia-solution added. The following details will show how dependent is the development upon the proximity to the mouth of the tube by which the ammonia-solution is applied.

No. 1 (furthest from the tube), one stem strong and vigorous at the base, and 13 inches high. No. 2 (third from the tube), three stems ; one 12 to 13 inches high, the others smaller. No. 3 (second from the tube), five stems ; three 12 to 13 inches high, and two new ones 1 to 2 inches high. No. 4 (nearest the tube), seven stems ; four 8 to 13 inches high, and three new ones 1 to 3 inches high.

All upper leaves green and vigorous, most of the lower yellow and dead ; some of the shoots entirely green.

August 10.—Tenth pipette-ful of ammonia-solution added.

August 17.—Eleventh pipette-ful of ammonia-solution added (a new solution, the pipette-ful =0·00359 gramme N.).

Recently, much more disposition to form stem ; several plants from 18 to 30 inches high ; nodes clear of the sheaths. Comparing with the Wheat without ammonia, which also tends more to stem of late, it appears that the amount of growth is due to the supply of combined nitrogen, and its character (stemmy) more to the season.

August 26.—Twelfth pipette-ful of ammonia-solution added.

September 7.—Thirteenth pipette-ful of ammonia-solution (the last application ; in all, 0·0508 gramme N.) added. Growth from each seed as under :—

No. 1 (furthest from the ammonia-solution tube), one stem 23 inches high ; twelve leaves ; two nodes clear ; slight indication of heading.

No. 2 (third from the tube), one main stem growing, and two shoots dead ; main stem 23 inches high ; three upper leaves green, six lower ones yellowish ; not heading yet.

No. 3 (second from the tube), three stems ; two like "No. 2" ; the third 12 to 14 inches high ; little tendency to form stem, but long leaves from the axis.

No. 4 (nearest the tube, some of the roots washed when the solution, or water, is

added), two stems and five small shoots :—(*a*) highest leaf touches the top of the shade, and 3 inches of it lie against the wet glass, by which it is injured ; ten leaves ; three bare nodes ; (*b*) 23 inches high ; nine leaves, three upper green, others yellowish ; nodes not clear of the sheath ; not heading yet ; (*c*) three small stems from the base, 6 to 8 inches high, ceased to grow, and apparently dying ; (*d*) two small rudimentary shoots ; ceased to grow, and dying.

Main stems—lower leaves yellow or dead ; those starting a few inches from the soil numerous, and 12 to 16 inches long ; those higher up, 4 to 6 inches long, green, and healthy ; apparently incapable of supporting all the shoots started.

October 5.—Plants principally increasing in height of stem ; three touch the top of the shade ; upper leaves green. Shaded from the direct sun to prevent injury from the little aqueous lenses formed on the interior of the shade ; yet sun apparently wanted for ripening.

October 24.—Shade entirely full of vegetable matter, some stems touching the top, and leaves touching on all sides. Season for growth about over ; plants seemed stationary during some recent cold weather, but, the last few days being warmer, they have revived again. [This remark applies to all the Cereals.]

October 26.—*Plants taken up ;* produce from each seed as under :—

No. 1 (furthest from the ammonia-solution tube), a mass of tufted leaves at the base ; five leaves higher up below any visible node, formed before the plants began to run up stem (it was the same with the other plants, and with the leafy growth last year) ; higher up five more leaves, four visible nodes, and a head ; total height 30 inches ; rachis $1\frac{1}{2}$ inch ; three barren joints, and six with unripe seeds.

No. 2 (third from the tube), two dead shoots at the base, with several leaves each ; twelve leaves higher up on the main stem, lower ones 6 to 9 inches long, upper ones shorter ; four nodes visible ; total height 28 inches ; rachis $1\frac{1}{2}$ inch long, three barren joints, and six with glumes and pales, but still green.

No. 3 (second from the tube), three stems : (*a*) 6 inches high ; ten long narrow leaves ; stem dead. (*b*) 24 inches high ; fourteen leaves below the first node, and three higher up ; two nodes visible ; plant still growing and vigorous. (*c*) Height 31 inches ; twelve leaves below the first node, and three above it ; swelled at the top with a head not yet out.

No. 4 (nearest the tube), seven plants—five being small shoots 2 to 8 inches high, and two main stems. As mentioned in respect to No. 1, and applicable pretty generally to the Cereals with ammonia, a dense matted mass of leaves below the first node near the base, 8, 12, and 15 inches long, with thick sheaths forming a dense coat at the base of the stem. These plants are individually as follow :—(*a*) 28 inches high ; three nodes ; rachis two inches long, with five barren joints, and seven with glumes and pales, and seeds forming, green. (*b*) 35 inches high ; four visible nodes ; rachis $2\frac{1}{2}$ inches long, with five barren joints and seven with glumes and pales, and shrivelled seeds turning yellow.

The soil wet, soft, and loose, and not filling up the interstices among the flints.

The roots tolerably distributed throughout the whole pot, though not extending much to the sides, but passing through all the holes at the bottom, and some at the sides, of the pot, and forming a dense matted mass underneath it in the pan. Those of the plants nearest to the watering and manuring tube have sent by far the most roots downwards into the pan,—those furthest from it having their roots proportionally much more confined in the soil near the base of the plant, where they are much matted, with divaricate branches and short branchlets—these characters being more nearly those of the Cereals grown without nitrogenous manure.

Preparation and analysis as described at pp. 543, 544.

No. 10.—*Barley* (1858); *four seeds; prepared soil; with nitrogenous manure.*
(See Plate XV. fig. 11.)

April 27.—Seeds set, and the pot placed over sulphuric acid, and covered with a glass shade.

May 7.—The pot removed to its shade on the stand.

May 20.—Two plants up.

May 22.—A pipette-ful of the sulphate-of-ammonia solution (=0·004 gramme N.) added to the soil.

May 29.—Two plants; 4 to 6 inches high; five leaves on each, the lowest dead, the others green; improved since the addition of ammonia, more active growth and colour deeper green. A pipette-ful of the phosphate-solution added.

June 7.—Second pipette-ful of the sulphate-of-ammonia solution added; also a pipette-ful of the phosphate-solution.

June 19.—Two plants; 10 to 12 inches high, forming stem well; each plant seven leaves; one stem has a small shoot forming at its base.

June 21.—Third pipette-ful of the sulphate-of-ammonia solution added.

June 26.—Fourth pipette-ful of the sulphate-of-ammonia solution added. Two plants; 16 to 18 inches high, healthy and vigorous; eight leaves each; also each a vigorously growing shoot 4 to 5 inches long.

July 3.—Fifth pipette-ful of the sulphate-of-ammonia solution added; also a pipette-ful of the phosphate-solution.

July 12.—Sixth pipette-ful of the sulphate-of-ammonia solution added.

July 14.—Seventh pipette-ful of the sulphate-of-ammonia solution added. Two plants, namely,—

No. 1. (*a*) Main stem, 26 inches high; lower leaves ripe or dead, and yellow, upper ones green and growing; beard just appearing at the head. (*b*) Shoot, 18 inches high; three ripe or dead leaves, and three green.

No. 2. (*a*) Main stem, 28 inches high; lower leaves ripe or dead, four upper ones green; air-roots developing. (*b*) Shoot, 12 to 14 inches high; one dead, and four green leaves. (*c*) Shoot, 4 to 6 inches high; one dead, and three green leaves.

July 19.—Eighth pipette-ful of the sulphate-of-ammonia solution added.

July 28.—Ninth pipette-ful of the sulphate-of-ammonia solution added.

July 29.—Only two of the seeds germinated; plants as follow:—

No. 1. (*a*) Main stem, out of the ground single, and then gives off (*b*) and (*c*); is 22 inches high; lower leaves dead, and lowest shows a fungous growth; six air-roots (two still growing) from first joint or point of separation; five nodes, each giving a leaf, only the top one green; head bursting forth. (*b*) 28 inches high; five nodes, with leaves, upper two only growing; stem slim below and thicker higher up; awns of head appearing. (*c*) 5 inches high; two leaves; fresh and green.

No. 2. More vigorous than No. 1, and livelier colour; leaves the ground single; half an inch up divides, and also gives off roots which go into the soil; three-quarters of an inch higher a second shoot, and more roots given off; one reaches the soil, two growing downwards, and several withered; lowest leaves dead and show fungous growth, particularly where they lie on the soil.

August 17.—Tenth pipette-ful of the ammonia-solution added (new solution =0·00359 N.). Plants growing vigorously under the influence of the ammonia; nearly at the top of the shade; several heads appearing.

August 24.—Eleventh pipette-ful of the ammonia-solution added.

September 7.—Twelfth pipette-ful of the ammonia-solution added.

October 5.—Plants ripening; seven stems with heads; lower leaves of each dead or ripe, and two or three upper ones of each green. Some new shoots appearing at the base.

October 24.—Three heads green, the others ripe.

October 26.—*Plants taken up*:—

Only two seeds grew, and gave plants as follow:—

No. 1. Stem divided a little above the surface of the soil, giving plant (*a*), and an inch higher up divides again, giving (*b*) and (*c*); below the first point of separation the stem scarcely thicker than a pin, hard and solid. (*a*) 34 inches high; six visible nodes; stem below the lowest very thin and hard, but larger, soft and succulent higher up; head 3 inches long, having sixteen joints, with glumes and pales, and awns 4 to 6 inches long; two ripe plump seeds, and others shrivelled up. (*b*) 28 inches high; five nodes; below the lowest stem hard, firm, dry, and almost solid, and but little thicker than a pin; stem higher up larger, but still quite delicate; head $1\frac{1}{2}$ inch long, with three joints barren, and seven with glumes, pales, and long awns, but no seed. (*c*) 30 inches high; five nodes; lower part of stem not quite so thick as (*a*) and (*b*); head ripe, rachis $2\frac{1}{2}$ inches long, with two joints barren, and thirteen with glumes, pales, and awns; also some shrivelled seeds.

No. 2. Stem to $1\frac{1}{2}$ inch above the soil little thicker than a pin, quite solid and firm; then a thick and bunchy node and six stems. Stem (*a*) 18 inches high to rachis; four nodes; four leaves; crooked rachis $1\frac{1}{4}$ inch long, with three joints barren, and seven with glumes, pales, and awns. (*b*) 21 inches high to head; rachis $2\frac{1}{2}$ inches long, with three joints barren, and thirteen with glumes, pales, and long awns; four nodes; five

leaves. (*c*) green ; $2\frac{1}{2}$ inches high, and still growing. (*d*), (*e*), and (*f*) about 25 inches high to rachis ; each with four or five nodes ; rachis $1\frac{1}{2}$ inch long, with three or four joints barren, and nine or ten with glumes, pales, and some green seeds.

The Root-development of these Barley plants was very extraordinary. Plant No. 1 has about ten roots coming from the first node or point of separation, extending deep into the soil, and some even through the bottom of the pot ; roots also come out from the next joint above, on each of the separate stems, but these do not reach the soil. Plant No. 2, from the first joint, whence spring the six stems, throws out more than a dozen small roots, five of which reach the soil and ramify in it, some of the ramifications going down into the pan. The roots starting within the soil not so much matted near the base of the stem as those of the Wheat ; a good many go down and ramify amongst the flints, or go through into the pan; they are finer, and not so much blunted and divaricated as the Wheat roots. For illustration of the curious root-development of the barley plant, see Plate XV. fig. 16.

Soil dry, loose, and porous.

Preparation and analysis as described at pp. 543, 544.

No. 11.—*Oats* (1858) ; *four seeds ; prepared soil ; with nitrogenous manure.*

(See Plate XV. fig. 12.)

April 27.—Seeds set, and pot placed over sulphuric acid, and covered with a glass shade.

May 7.—The pot removed to its place on the stand.

May 20.—Three plants up ; 4 to $4\frac{1}{2}$ inches high ; two leaves each ; green and healthy.

May 22.—A pipette-ful of the sulphate-of-ammonia solution added (=0·004 grm. N.).

May 29.—Three plants ; 5 to 7 inches high ; four or five leaves each ; two lowest dead, three uppermost green and growing. These plants have increased the most of any since the addition of ammonia. A pipette-ful of the phosphate-solution added.

June 7.—Second pipette-ful of the sulphate-of-ammonia solution added ; also a pipette-ful of the phosphate-solution.

June 19.—Three plants ; 10 to 14 inches high ; six leaves each ; apparently somewhat injured by hot sun the last few days.

June 21.—Third pipette-ful of the sulphate-of-ammonia solution added.

June 26.—Fourth pipette-ful of the sulphate-of-ammonia solution added. Three plants, 12 to 14 inches high. No. 1, head developed and obviously seeds forming ; one shoot 1 inch, and one 4 inches long from the base. No. 2, head forming ; two shoots from the base 4 to 5 inches long. No. 3, much like No. 2. Growth of main stems apparently checked and that of shoots increased by some recent hot weather ; in all, six shoots ; green, healthy, and promising further growth.

July 3.—Fifth pipette-ful of the sulphate-of-ammonia solution, also a pipette-ful of the phosphate-solution added.

July 12.—Sixth pipette-ful of the sulphate-of-ammonia solution added.

July 14.—Seventh pipette-ful of the sulphate-of-ammonia solution added. Plants as follow:—

No. 1. Two stems; one ripe, with two or three seeds; six leaves, lowest dead ripe, and five green. The other stem 12 to 14 inches high; green; forming head.

No. 2. Three stems, from 12 to 15 inches high. One stem swelled at the top with a head, but not maturing; checked early, apparently from want of available nitrogen, after which the ammonia added developed the two shoots, whose stems are green, with one dead and four green leaves, and heads forming.

No. 3. Two stems; one 8 to 10 inches high, dead or ripe; the other 14 to 15 inches high, green and healthy.

July 30.—*The plants taken up*:—

The experiment stopped rather prematurely, the shade getting slightly cracked. The plants had manifested three orders of growth, as follow:—

(1) The first growth, which was forming head when a fresh supply of ammonia gave rise to shoots at the base. (2) The above shoots gave three headed stems: one with three full and two imperfect seeds; one with two full and one imperfect seed; and one with one full and two imperfect seeds. (3) New shoots from the base on the further addition of ammonia; green and healthy at the close, but promising to go to seed.

The roots were very little more distributed than those of the Oats without nitrogenous manure in No. 3 shade, and only extended about half the depth of the pot. The soil was wet and soft.

Preparation and analysis as described at pp. 543, 544.

No. 12.—*Beans* (1858); *three seeds; prepared soil; with nitrogenous manure.*

April 27.—Seeds set, and the pot placed over sulphuric acid, and covered with a glass shade.

May 20.—Pot removed to its shade on the stand. Three plants up; $2\frac{1}{2}$ inches high; three leaves on each, colour dark green; healthy.

May 22.—A pipette-ful of the sulphuric-acid solution added.

May 29.—Three plants; one dead, one much specked, the third improving. A pipette-ful of the phosphate-solution added.

June 7.—A pipette-ful of the sulphate-of-ammonia solution added; also a pipette-ful of the phosphate-solution.

June 19.—All apparently dying; leaves much specked with black.

June 21.—Plants all obviously past recovery; removed but not analysed.

No. 13.—*Peas* (1858); *three seeds; prepared soil; with nitrogenous manure.*

April 27.—Seeds set, and the pot placed over sulphuric acid, and covered with a glass shade.

May 20.—Pot removed to its shade on the stand. Three plants up, growing exceedingly well; 3, $2\frac{1}{2}$, and 1 inch high; three, two, and two leaves, respectively.

May 22.—A pipette-ful of the sulphuric-acid solution added.

May 29.—One plant dead; another sickly; the third has given off shoots 3 to 4 inches high, which appear healthy. A pipette-ful of the phosphate-solution added.

June 7.—A pipette-ful of the sulphate-of-ammonia solution (=0·004 gramme N.) added; also a pipette-ful of the phosphate-solution.

June 19.—Two plants dead; the third has two green shoots, 3 and 5 inches high, but delicate.

June 26.—The two shoots growing languidly; seven leaves each; also some leaflets.

July 3.—A pipette-ful of the phosphate-solution added.

July 14.—Main shoot 12 to 13 inches high; four lower leaves dead, four upper green; two shoots at the base dead, and one green and thriving.

July 29.—The two dead plants entirely prostrate, and near one a pale-green moss formed on the surface of the soil. The third plant has three dead branches, and one about 6 inches high with green leaves at the top.

August 17.—All the plants dead.

August 24.—Plants taken up, and submitted to analysis, although the growth so unsatisfactory. Of course, of themselves, the results can have little weight in reference to the question at issue.

No. 14.—*Clover* (1858); 226 *seeds; prepared soil; with nitrogenous manure.*

Two pots of Clover had been sown at the same time as the other plants, one to be without, and the other with nitrogenous manure. They came up well, but very soon died; and on June 6 two more pots were sown in the same manner as before, excepting that the ash was neutralized with sulphuric acid, and re-ignited before being mixed with the soil; both also had a pipette-ful of the phosphate-solution, and the one that was to have nitrogenous manure a pipette-ful of the ammonia-solution, at the time of sowing the seed. The one sown without the ammonia-solution failed so soon that the experiment was abandoned early; the other (with the ammonia) forms the subject of the following record.

June 6.—Seeds set as above, in soil with neutralized ash, and both phosphate and sulphate-of-ammonia solution added*.

June 19.—A good deal up; actively and healthily growing.

June 21.—Second pipette-ful of the sulphate-of-ammonia solution added*.

June 26.—Third pipette-ful of the sulphate-of-ammonia solution added. Plants quite green and healthy.

July 3.—Fourth pipette-ful of the sulphate-of-ammonia solution added; also a pipette-ful of the phosphate-solution.

July 12.—Fifth pipette-ful of the sulphate-of-ammonia solution added.

July 14.—Sixth pipette-ful of the sulphate-of-ammonia solution added. Surface of

* It is not quite certain that the ammonia-solution was added at this date; and in the Table of the results (XIV. p. 531) it is assumed that it was not.

the soil covered with plants, growing well; 3 to 4 inches high, with two or three trifoliate leaves each.

July 19.—Seventh pipette-ful of the sulphate-of-ammonia solution added.

July 28.—Eighth pipette-ful of the sulphate-of-ammonia solution added.

July 29.—The plants growing, but not vigorously; some black and dead, and some withering leaves; some petioles more than 4 inches long.

August 10.—Ninth pipette-ful of the sulphate-of-ammonia solution added.

August 17.—Tenth pipette-ful of the sulphate-of-ammonia solution added (new solution = 0·00359 gramme N.). Green and growing well; twenty to twenty-five stems 4 to 6 inches high; a good many roots above ground.

September 7.—Eleventh pipette-ful of the sulphate-of-ammonia solution added. Large strong roots visible; numerous leaves from the base; not much development of stem; length of petioles 3 to 5 inches.

October 5.—Twelfth pipette-ful of the sulphate-of-ammonia solution added. Not growing much, yet not dying; stems becoming bushy.

October 24.—Many of the lower petioles and leaves dead, and some a little mouldy; from the base of the dead petioles generally spring a number of green leaves, and petioles 2 to 4 inches long bearing green leaves, which look healthy, but almost stationary from day to day.

October 26.—*Plants taken up* :—

Twenty-one plants; much as at last date.

Roots somewhat matted at the base, and throughout the surface of the soil; extending 2 to 3 inches through the soil, and some to the inner sides of the pot; none among the flints, or in the pan under the pot.

Soil rather wet, loose, and porous.

Preparation and analysis as described at pp. 543, 544.

No. 15 (1858).—*Wheat, Barley, Oats, Beans, Peas, and Clover, in a pot of Garden soil.*

May 20.—Some of the plants just coming up.

May 29.—Wheat, Barley, and Oats, 6 to 8 inches high; Beans 4 to 6 inches high, with some specks on the leaves; Clover up and growing. The condensed water yellowish, and gives a yellowish-green deposit.

June 19.—The pot full of green herbage. Wheat, Barley, Oats, and Beans, all healthy and growing well; Peas 3 to 4 inches high; Clover 2 to 3 inches, with three leaflets.

June 26.—Wheat and Barley touch the top of the shade, and the plants crowded; Clover 3 to 4 inches high.

July 14.—Shade full of growing matter. Wheat and Barley just heading.

August 2.—The Oat heading, with two grains; Wheat growing, but the ends of some of the leaves dying. The two Beans quite black and dead. Several kinds of weed come up; two in bloom.

August 17.—Shade full of vegetable matter. Wheat, Barley, Oats, and weeds growing; but Leguminous plants dead.

September 7.—A good deal of growth yet.

October 5.—A good deal of grass and weeds growing. Cereals ripening.

October 24.—Shade full; experiment stopped. Wheat not quite ripe. Barley and Oats dead ripe; Beans and peas all dead; a little Clover still living; some grass, and other weeds, green, and some seeded. The whole soil filled with roots, many distributed through the flints, and a large quantity growing in the pan under the pot.

No. 16.—*Buckwheat* (1858); *forty-two seeds* (1 *gramme*); *prepared soil; with nitrogenous manure.*

August 20.—Seed sown, and the pot placed over sulphuric acid, and covered with a glass shade.

August 24.—Pot removed to its shade on the stand. Several plants up.

September 7.—A pipette-ful of the sulphate-of-ammonia solution added (=0·00359 gramme N.). Plants growing well.

October 5.—Second pipette-ful of the sulphate-of-ammonia solution added. Much more vigorous than the Buckwheat without ammonia (No. 7); about twenty plants; 5 to 7 inches high; four to six leaves on each.

October 24.—Third pipette-ful of the sulphate-of-ammonia solution added. Growing well; 5 to 7 inches high; six plants in bloom. Comparing with No. 7, the influence of ammonia here is very marked, as shown in size, vigour, and maturation.

November 22.—*Plants taken up*:—

Twenty-four plants; 4 to 7 inches high; five to seven leaves on each; six stems have flowered; bloom gone off and rudimentary seed formed; would probably have grown more but for frost.

Roots less in proportion to upward growth than with Buckwheat without ammonia; none deeper in the soil than $1\frac{1}{2}$ to 2 inches; slim, delicate, and but little distributed.

Soil loose and porous.

Preparation and analysis as described at pp. 543, 544.

No. 17 (1858).—Plants grown with Nitrogenous Manure in M. G. Ville's Case.

The same descriptions of pot, pan, soil, ash, &c., were used for these experiments as for the others, as explained at page 565. Plants as under :—

Wheat, four seeds; Barley, four seeds; Oats, four seeds; Beans, three seeds.

June 29.—Seeds set, and the pots placed over sulphuric acid, and covered with a glass shade.

July 5.—Pots removed to M. Ville's Case.

July 14.—A pipette-ful of the sulphate-of-ammonia solution given to each pot (=0·004 gramme N.). Wheat, four plants just appearing; Barley, three plants $1\frac{1}{2}$ inch

high ; Oats, two plants just appearing ; Beans, one plant 4 inches high, two others just appearing.

July 19.—Second pipette-ful of the sulphate-of-ammonia solution to each pot.

July 28.—Third pipette-ful of the sulphate-of-ammonia solution to each pot of Cereals.

August 2.—Wheat, four plants ; 7 inches high ; extremities of some of the leaves dying. Barley, three plants ; two 9 inches, and one 7 inches high ; two lower leaves of each yellow, remainder green. Oats, two plants ; 10 inches high ; the lower leaf of each dead, and the tips of some of the others turning yellow. Beans, two plants up ; one 8, and the other 6 inches high.

August 17.—Fourth pipette-ful of the sulphate-of-ammonia solution added (new solution, =0·00359 gramme N.). All the plants growing luxuriantly. Wheat, about 14 inches high ; Barley and Oats, 8 to 12 inches high ; Beans, 9 and 12 inches high.

September 7.—Fifth pipette-ful of the sulphate-of-ammonia solution added.

Wheat, four plants ; (*a*) 14 inches high with ten leaves ; (*b*) 13 inches high with nine leaves ; (*c*) 15 inches high with ten leaves ; (*d*) 11 inches high with ten leaves.

Barley, three plants ; (*a*) 23 inches high with eleven leaves ; (*b*) 14 inches high with ten leaves ; (*c*) 25 inches high with eleven leaves.

Oats, two plants ; (*a*) 26 inches high with seven leaves ; (*b*) 29 inches high, nine leaves.

Beans, two plants ; main stems dead, but new shoots growing.

October 5.—Wheat, four healthy and vigorous plants ; (*a*) 14 inches high ; two green and eight dead leaves ; (*b*) 14 inches high ; two green and seven dead leaves ; (*c*) 15 inches high ; two green and eight dead leaves ; (*d*) 14 inches high ; three green and eight dead leaves.

Barley, three plants ; two healthy and vigorous, and one less so ; (*a*) 24 inches high ; three green and eight dead leaves ; (*b*) 18 inches high ; three green and seven dead leaves ; (*c*) 28 inches high ; three green and eight dead leaves.

Oats, two healthy and vigorous plants, in ear ; (*a*) 30 inches high ; two green and five dead leaves ; (*b*) 30 inches high ; two green and seven dead leaves.

Beans, two plants ; (*a*) 9 inches high with three branches ; (*b*) 14 inches high with three branches.

October 24.—Wheat, four plants, erect and firm ; much as on October 5, but larger ; no nodes visible ; but little tendency to form stem. Barley, much as on October 5. Oats, much as on October 5. Beans, apparently ceased to grow.

Sixth pipette-ful of the sulphate-of-ammonia solution added. Wheat, four plants, 14 to 18 inches high ; stems stout and erect, but without visible nodes ; very leafy. Barley, four plants, just coming to ear. Oats, two plants, main stems touching the top of the Case ; (*a*) has a shoot 22 inches long springing from the first node 2 inches from the soil, and coming into head ; (*b*) has three small shoots, one $\frac{1}{2}$ inch and one $1\frac{1}{2}$ inch long, springing from the base, and one 3 inches long from the first node. Beans, slightly revived since the last report.

Notes on taking up the Plants.

Wheat taken up December 9.—The four plants all about the same size ; strong, healthy, and vigorous ; ten to twelve long dead leaves each, 8, 10, or 12 inches long, closely compacted one above another at their base, and two or three green flourishing leaves each at the top. Roots very thick and matted at the base, extending immediately around in all directions, with short rootlets covered with divaricated branches, filling the upper layers of soil ; not many extend more than $2\frac{1}{2}$ inches down, very few go through the bottom of the pot into the pan, and none ramify there. Soil loose, porous, and moist ; and some water in the pan.

Barley taken up December 9.—Three plants ; (*a*) 29 inches high, with a small shoot (second growth) 1 inch long, from the base ; fifteen leaves, two top ones green and growing ; swelled at the top with a head forming ; (*b*) 20 inches high ; thirteen leaves, upper ones green and growing ; and indications of head forming ; (*c*) much like (*a*) in height, leaves, and shoot ; a short air-root from the first node. Roots the same character in all ; branched, considerably matted at the base, finely divided with numerous little divaricate branchlets ; only a few extend down into the pan, and they do not ramify in it. Soil loose, open, porous, and moist ; some water in the pan.

Oats taken up December 9.—Two plants :—(*a*) with two stems ; one 32 inches high, eleven imperfect seeds, and seven leaves ; the other 26 inches high, coming from the second node 2 inches up the main stem, with twelve seeds ; six or eight air-roots 1 to 2 inches long, coming from the base node ; a shoot an inch long from the lowest node, and another lower down at the base. (*b*) A main stem with two long branched ones ; main stem 32 inches long, with five leaves and ten undeveloped seeds without solid nucleus ; one branched stem, from the third node, 3 inches up the main stem, 14 inches long, with three leaves, still green and growing ; the other branch from the base of the main stem 10 inches long, green and growing ; some air-roots 1 to 2 inches long come from the base of the largest branch, also several from the next node below, one or two of which extend to the soil and ramify in it. There is also another small branch 2 to 3 inches long coming from the base of the plant and still green and growing. Roots much alike in both ; considerably matted at the base, little distributed, extending 3 or 4 inches in the upper parts of the soil, with many small divaricate branches ; no roots in the pan. Soil loose, porous, and moist ; and some water in the pan.

Beans taken up December 9.—Two plants, with eight or ten leaves each ; one 9 inches, the other 14 inches high ; both dead. The roots of both very much branched at the base of the stem, but not extending deep into the pot, none going down to the flints.

The Wheat, Barley, Oats, and Beans, prepared and analysed as described at pp. 543, 544.

Fig 1
Plan of Pot

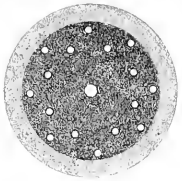


Fig 2
Elevation of Pot

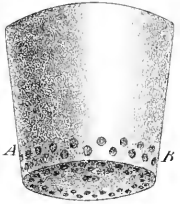


Fig 3
Elevation of Pot & Pan

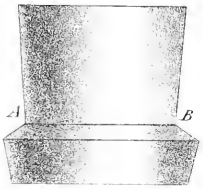
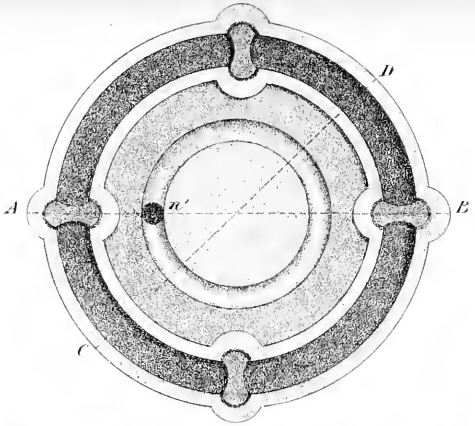
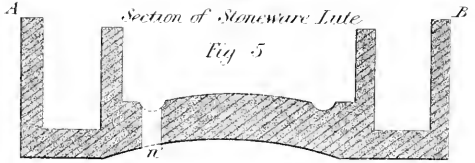


Fig 4
Plan of Stoneware Lute



Section of Stoneware Lute
Fig 5



Section of Stoneware Lute
Fig 6.

Scale 4 inch to 1 inch

Fig 7

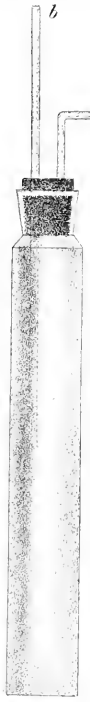
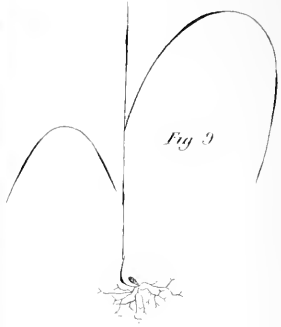


Fig 9



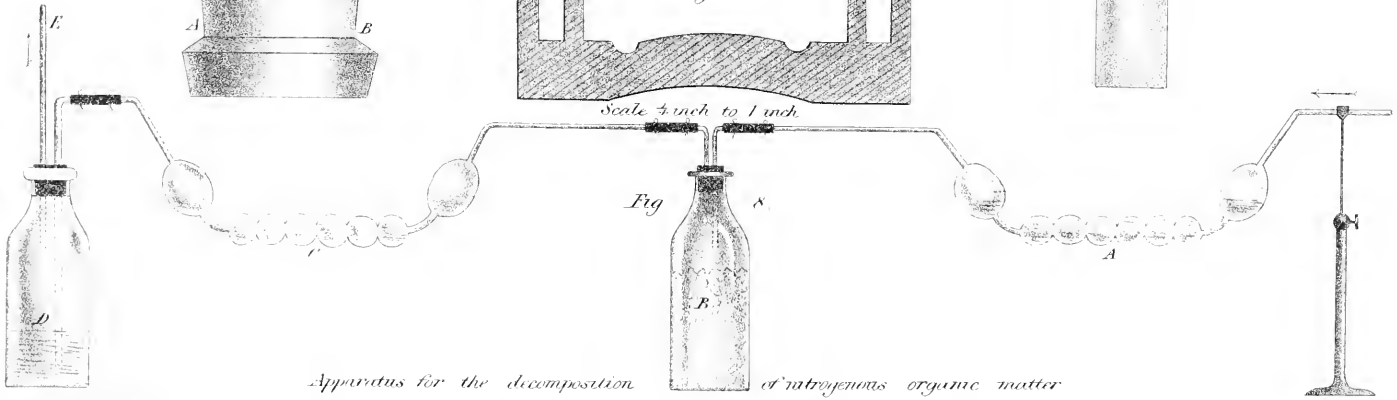
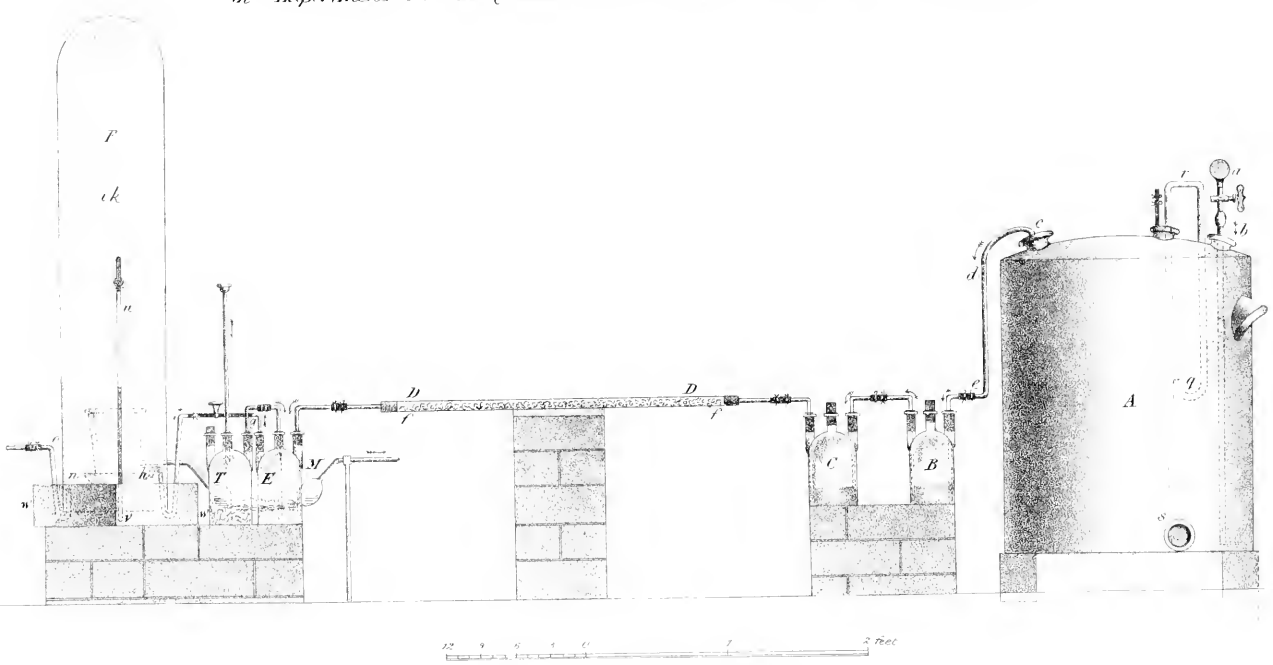


Fig 8

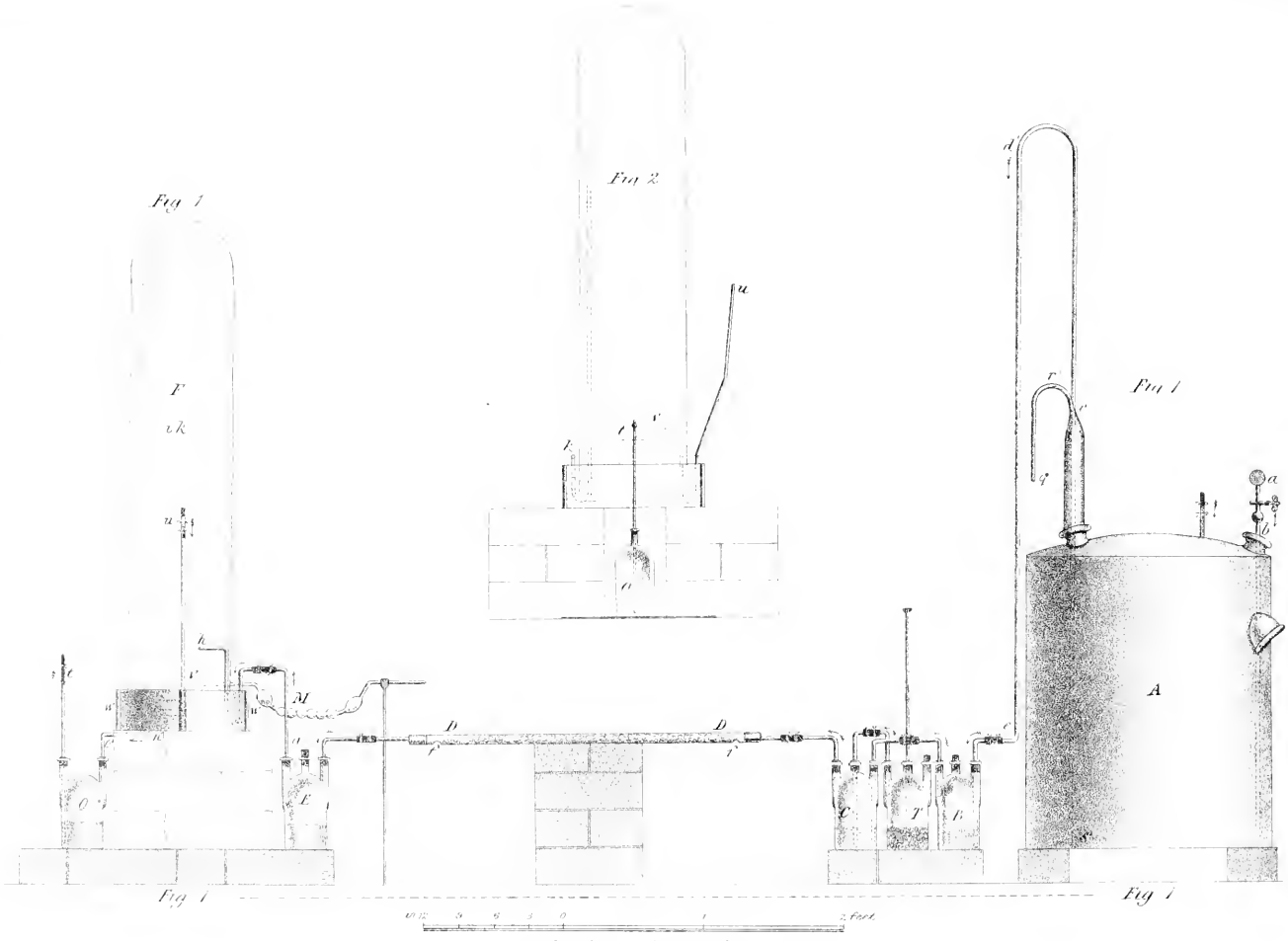
Apparatus for the decomposition of nitrogenous organic matter

APPARATUS USED IN 1857

in Experiments on the Question whether Plants assimilate Free Nitrogen



Scale of 1 inch to a foot



Scale of 1 inch to a foot

Apparatus used in 1858, in Experiments on the Question whether Plants assimilate Free Nitrogen

Supplied with no other combined Nitrogen than that contained in the seed sown

Fig 1
Wheat — 1857
in prepared soil

Fig 2
Barley — 1857
in prepared soil

Fig 3
Barley — 1857
in prepared pumice

Fig 4
Wheat — 1858
in prepared soil

Fig 5
Barley — 1858
in prepared soil

Fig 6
Oats — 1858
in prepared soil

Supplied with known and limited quantities of combined Nitrogen beyond that contained in the seed sown

Fig 7
Wheat — 1857
in prepared soil

Fig 8
Barley — 1857
in prepared soil

Fig 9
Barley — 1857
in prepared pumice

Fig 10
Wheat — 1858
in prepared soil

Fig 11
Barley — 1858
in prepared soil

Fig 12
Oats — 1858
in prepared soil

Fig 13
Wheat Barley & Beans — 1857
in Garden soil

Fig 14

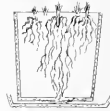


Fig 15

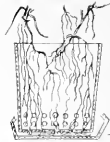


Fig 16

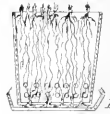


Fig 17

Scale 1½ inch to a foot

PHILOSOPHICAL TRANSACTIONS.

I. *On the present Position of the Question of the Sources of the Nitrogen of Vegetation, with some new Results, and preliminary Notice of new Lines of Investigation.*

By Sir J. B. Lawes, *Bart., LL.D., F.R.S., and Professor* J. H. Gilbert, *LL.D., F.R.S.*

Received, Part 1, July 20, 1887; Parts 2 and 3, May 3, 1888—Read May 17, 1888.

Contents.

Page.

Introduction 2

Part I.

Results relating to other Sources than Free Nitrogen.

1. Summary of previously published Rothamsted Results, chiefly relating to Nitric Acid in Soils and Subsoils 3
2. New determinations of Nitric Acid in Soils and Subsoils 9
3. Percentage of Nitrogen in the Surface Soils of the Experimental Plots 13
4. Experiments on the Growth of Red Clover on Bean-exhausted Land 18
5. Experiments on the Nitrification of the Nitrogen of Subsoils 22
6. Can Roots, by virtue of their Acid Sap, attack, and render available, the otherwise insoluble Nitrogen of the Subsoil? 27
7. Action of dilute Organic Acid Solutions on the Nitrogen of Soils and Subsoils 29
8. Evidence as to whether Chlorophyllous Plants can take up Complex Nitrogenous Bodies, and assimilate their Nitrogen 38

Part II.

Recent Results and Conclusions of others, relating to the Fixation of Free Nitrogen.

1. The Experiments of M. Berthelot 43
2. The Experiments of M. Dehérain 48
3. The Experiments of M. Joulie 53

4. The Experiments of Dr. B. E. Dietzell 58
5. The Experiments of Professor B. Frank 60
6. The Experiments of Professor Hellriegel, and Dr. Wilfarth 64
7. The Experiments of Professor Emil von Wolff 71
8. The Experiments of Professor W. O. Atwater 79
9. Recent Results and Conclusions of M. Boussingault 83

Part III.

Summary, and General Considerations and Conclusions.

1. The Evidence relating to other Sources than Free Nitrogen 89
2. The Evidence relating to the Fixation of Free Nitrogen 94
3. General Considerations and Conclusions 100

Postscript 107

Introduction.

A great part of this paper was written in the spring of 1886, but its completion was unavoidably delayed. This has, however, not been altogether without advantage. Thus, in the first place, at the *Naturforscher-Versammlung*, held in Berlin, in September, 1886, the greater part of the sittings of two days was devoted, in the Section of *Landwirthschaftliches Versuchs-Wesen*, to the discussion of the subject from various points of view, one of ourselves taking part; and as it seemed desirable that the results and conclusions then brought forward by others should be considered, we have waited for the publication of the exact figures in some cases. Again, since the Berlin meeting, M. Berthelot has published some further results, to which reference should be made. And lastly, we are now enabled to give further new results of our own.

In Part 2 of the 'Philosophical Transactions' for 1861, a paper was given, by ourselves and the late Dr. Pugh, "*On the Sources of the Nitrogen of Vegetation, with special reference to the question whether plants assimilate free or uncombined Nitrogen.*" Since that time, the question of the sources of the nitrogen of vegetation has continued to be the subject of much discussion, and also of much experimental enquiry, both at Rothamsted and elsewhere. Until quite recently, the controversy has chiefly been as to whether plants directly assimilate the free nitrogen of the atmosphere; but, during the last few years, the discussion has assumed a somewhat different aspect. The question still is whether the free nitrogen of the air is an important source of the nitrogen of vegetation; but whilst few now adhere to the view that the higher chlorophyllous plants directly assimilate free nitrogen, it is, nevertheless, assumed to be brought under contribution in various ways—coming into combination within the soil, under the influence of electricity, or of micro-organisms, or of other low forms, and so indirectly serving as an important source of the nitrogen

of plants of a higher order. Several of the more important of the investigations in the lines here indicated seem to have been instigated by the assumption that compensation must be found for the losses of combined nitrogen which the soil sustains by the removal of crops, and also for the losses which result from the liberation of nitrogen from its combinations under various circumstances.

At the meeting of the American Association for the Advancement of Science, held at Montreal in 1882, we gave a paper entitled—"*Determinations of Nitrogen in the soils of some of the Experimental Fields at Rothamsted, and the bearing of the results on the question of the Sources of the Nitrogen of our crops;*" and again, at the Meeting of the British Association, held at Montreal, in 1884, we gave further results on the subject, in a paper—"*On some points in the Composition of Soils; with results illustrating the Sources of the Fertility of Manitoba Prairie Soils.*"*

It is the object of the present paper to summarise some of our own more recently published results bearing on various aspects of the subject, to put on record additional results, to give a preliminary notice of new lines of enquiry, and to discuss the evidence so adduced with reference to the results and conclusions of others which have recently been put forward, as above alluded to.

PART I.

Results relating to other Sources than Free Nitrogen.

1. *Summary of previously published Rothamsted Results chiefly relating to nitric acid in soils and subsoils.*

Before directing attention to the new results it will be desirable, with the view of bringing out their significance the more clearly, to give a brief *résumé* of our previous results and conclusions bearing on the subject.

In the last mentioned paper, after reviewing previously existing evidence as to the sources of the nitrogen of crops, we concluded, as we had done before, that, excepting the small amount of combined nitrogen annually coming down in rain, and the minor aqueous deposits from the atmosphere, the source of the nitrogen of vegetation was, substantially, the stores within the soil and subsoil, whether derived from previous accumulations or from recent supplies by manure.

Results of determinations of the nitrogen as nitric acid, in soils of known history as to manuring and cropping, and to a considerable depth, in some cases to 108 inches, were given, which showed that the amount of nitrogen in the soil in that form was much less after the growth of a crop than under corresponding conditions without a crop. In the case of gramineous crops, the evidence pointed to the conclusion that most, if not the whole, of their nitrogen was taken up as nitric acid from the soil and

* Afterwards revised and published in the 'Transactions of the Chemical Society' for June, 1885.

subsoil. In the case of leguminous crops again, the evidence was in favour of the supposition that, in some cases, the whole of the nitrogen had been taken up as nitric acid, but that in others that source was inadequate.

The results further showed that, under otherwise parallel conditions, there was very much more nitrogen as nitric acid, in soils and subsoils, down to a depth of 108 inches, where leguminous than where gramineous crops had for some time been grown.

Table II., p. 6, gives, in a condensed form, the most important of the previously published results relating to this branch of the subject. It shows the amounts of nitrogen as nitric acid, calculated *per acre*, in lbs., according to determinations made in samples of soil collected in 1883, at each of 12 depths of 9 inches each, that is down to 108 inches in all, under the following conditions :—

1. Where wheat had been grown in alternation with fallow, without any manure, since 1850 ; that is for more than 30 years.

2. On a plot where mineral manures had occasionally been applied, but no nitrogen for more than 30 years; where *Trifolium pratense* had been sown 12 times during the 30 years, 1848–77, but in 8 out of the last 10 trials the plant had died off in the winter or spring succeeding the sowing, in 4 without giving any crop, and in the other 4 yielding very small cuttings; and where, consequent on the failure of the clover, during the 30 years 1 crop of wheat and 5 of barley had been taken, and the land had been 12 years left fallow. *Trifolium repens* was then sown, namely, in 1878, 1880, 1881, and 1883; and it yielded crops in 1879, 1881, and 1882, but none in 1883, when the soil samples were taken.

3. On 2 plots with the same previous history as that of the *Trifolium repens* plots prior to 1878, but where *Vicia sativa* has since been sown; and where, notwithstanding the previous failure of the red clover, the *Vicia* yielded fair crops in 1878, 1879, 1880, and 1881, and large crops in 1882, and in 1883, before the soil samples were taken.

We are now able to give amended, and more complete estimates, of the amounts of nitrogen removed per acre in the produce from the different plots, both during the preliminary period up to 1877 inclusive, and during the period of the direct experiments with the various leguminous plants. As each leguminous plot is only $\frac{1}{8\frac{1}{4}}$ of an acre in area, it is obvious that calculations of the produce, per acre, can only be approximately correct. We have, therefore, in each case taken the average yield of three plots, 4, 5, and 6, with one and the same plant, but with somewhat different mineral manures. Thus, plot 4 has received a mixture of superphosphate of lime and potash sulphate ; plot 5 a mixture of potash, soda, and magnesia salts ; and plot 6 the same as plot 5, with superphospate of lime in addition. Direct determinations of nitrogen have, in almost all cases, been made ; and we believe that the estimates of it per acre may be considered as close approximations to the truth, and at any rate quite sufficiently so for the purposes of illustration and of argument for which they are used. The results are given in the following Table (I.).

TABLE I.—Estimated yield of Nitrogen per acre, in lbs., in wheat alternated with fallow, and in various leguminous crops without nitrogenous manure.

	Unmanured.	Mineral manures only.	
	Fallow-wheat.	*Trifolium repens.*	*Vicia sativa.*
Preliminary period:—Wheat and fallow, 27 years, 1851–77. Red clover, &c., &c., 29 years, 1849–77.			
	lbs.	lbs.	
Average per acre per annum . .	17	32	
Experimental period.			
	lbs.	lbs.	lbs.
1878	29	0	51
1879	Fallow	82	46
1880	24	0	58
1881	Fallow	8	65
1882	18	74	146
1883	Fallow	0	101
Total, 6 years	71	164	467
Average annual	12	27	78

The next Table (II.) shows the amounts of nitrogen as nitric acid found in the soils and subsoils of the several plots under the conditions above described.

TABLE II.—Nitrogen as Nitric Acid per acre, lbs., in the soils and subsoils of some experimental plots, without nitrogenous manure for more than 30 years. Hoosfield, Rothamsted. Soil samples collected July 17–26, 1883.

Depths.	Unmanured.	Series I. Mineral manures only.			*Trifolium repens.* + or −. Wheat land.	+ or −. *Trifolium repens.*	
	Wheat-fallow land.	*Trifolium repens.* Plot 4.	*Vicia sativa.* Plot 4.	*Vicia sativa.* Plot 6.		*Vicia sativa.* Plot 4.	*Vicia sativa.* Plot 6.
inches.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1– 9	19·85	30·90	12·16	10·22	+ 11·05	− 18·74	− 20·68
10– 18	8·05	27·73	4·11	2·72	+ 19·68	− 23·62	− 25·01
19– 27	2·47	8·44	1·37	1·08	+ 5·97	− 7·07	− 7·36
28– 36	2·70	7·64	1·67	1·52	+ 4·94	− 5·97	− 6·12
37– 45	1·62	9·07	4·58	2·51	+ 7·45	− 4·49	− 6·56
46– 54	3·57	8·77	6·37	4·42	+ 5·20	− 2·40	− 4·35
55– 63	3·84	7·92	7·16	4·52	+ 4·08	− 0·76	− 3·40
64– 72	2·28	8·34	5·95	4·92	+ 6·06	− 2·39	− 3·42
73– 81	1·48	8·27	4·54	4·81	+ 6·79	− 3·73	− 3·46
82– 90	1·76	9·95	5·32	5·14	+ 8·19	− 4·63	− 4·81
91– 99	2·94	9·16	5·66	6·40	+ 6·22	− 3·50	− 2·76
100–108	1·84	9·51	5·32	6·46	+ 7·67	− 4·19	− 3·05
Total	52·40	145·70	64·21	54·72	+ 93·30	− 81·49	− 90·98

These wheat-fallow and leguminous plots are absolutely adjoining; and by their previous treatment their surface soils had become extremely poor in nitrogen. The results have been discussed in detail in the paper in the Transactions of the Chemical Society, and must only be briefly summarised here. Table I. shows that, for about 30 years, the *Trifolium repens* soil had yielded in crops nearly twice as much nitrogen per acre as the wheat-fallow soil. Yet it is seen that, whilst the wheat-fallow soil contained, down to the depth of 9 feet, only 52·4 lbs. of nitrogen as nitric acid per acre, the *Trifolium repens* soil contained 145·7 lbs. to the same depth. In other words—the *Trifolium repens* soil, from which so much more nitrogen had been removed, contained 93·3 lbs. more nitrogen as nitric acid than the wheat-fallow soil.

Now, excepting that the leguminous crop soil had received mineral manures, and the wheat soil had not, the characteristic difference in the history of the two plots was, that the one had grown a gramineous crop alternately with fallow for more than 30 years, and the other had, during the same period, besides growing 6 gramineous crops, and being frequently fallow, been sown 12 times with red clover, and, during the immediately preceding 6 years, 4 times with white clover. That is to say, the chief distinction was, that the one plot had, especially in the earlier and the later years, grown a leguminous crop, whilst the other had not; and it is under these circumstances that the leguminous crop soil is found to contain, down to 108 inches, nearly 3 times as much nitrogen as nitric acid as the gramineous crop soil.

The difference is the greatest near the surface, but it is very considerable down to the lowest depth. Hence it is obvious that any loss by drainage would be much the greater from the *Trifolium* plot, and so the difference between the two plots was probably in reality greater than the figures show. In both cases the actual amount is the greatest near the surface, indicating more active nitrification; and the excess is much the greater in the *Trifolium repens* soil, doubtless due to more nitrogenous crop-residue from the leguminous than from the gramineous crop. Indeed, about 74 lbs. of nitrogen had been removed in the *Trifolium repens* crop in 1882, and none in 1883, the year of the soil collections. On the other hand, only about one-fourth as much was removed in the wheat crop of 1882, and the land was fallow in 1883. Unless, however, there was considerably more nitrogen in the crop-residue than in the removed crops of the *Trifolium repens*, the excess of 93 lbs. of nitrogen as nitric acid found in the *Trifolium repens* soil, together with the increased amount lost by drainage, could not have had its source entirely in the nitrification of recent nitrogenous crop-residue. Some of the increased amount in the lower layers was indeed doubtless due to washing down from the surface. But as, notwithstanding much more nitrogen had been removed in crops from the leguminous than from the gramineous crop soil during the previous 30 years, the surface soil of the leguminous plots remained slightly richer in nitrogen than that of the gramineous plot, it cannot be supposed that the whole of the nitrogen of the crop, and of the nitric acid found, had its origin in the surface soil. If, therefore, nitrogen has not been derived from the atmosphere, the conclusion must be that some has come from the subsoil.

The indication was that nitrification had been more active under the influence of the leguminous than of the gramineous growth and crop-residue. In fact, under the influence of leguminous growth, not only will there be increased nitrogenous matter for nitrification, but it would seem that the development of the nitrifying organisms will be favoured. The question is, therefore, whether part of the result be not due to the passage downwards of the nitrifying organisms, and the nitrification of the nitrogen of the subsoil.

The alternative was suggested, that the soil and subsoil might still be the source of the nitrogen of the crops, but that the plants may take up, at any rate part of their supply, in other forms than as nitric acid—as ammonia, or as organic nitrogen, for example. It was pointed out that fungi do take up both organic carbon and organic nitrogen; but that, whilst existing direct experimental evidence was conflicting as to whether green leaved plants even assimilate carbon taken up by their roots as carbonic acid, the evidence was even less conclusive as to whether they take up either organic carbon or organic nitrogen as such from the soil. To this question we shall recur further on.

The next point is to compare the amount of nitrogen as nitric acid found in the *Vicia sativa* soils with that in the *Trifolium repens* soil. In the first place it is to be observed, that whilst from the *Trifolium repens* plot only 164 lbs. of nitrogen had

been removed in the crops during the five years to 1882 inclusive, from the *Vicia sativa* plots 366 lbs. had been removed during the same period. Further, whilst from the *Trifolium repens* plot there was no nitrogen removed in 1883, the year of soil sampling, from the *Vicia* plots 101 lbs. were removed in the crop just before the soil sampling. It is seen that, under these circumstances, there remained, per acre, in one of the *Vicia* plots 81·5 lbs., and in the other 91 lbs., less nitrogen as nitric acid to the depth examined than in the *Trifolium repens* soil.

If we confine attention only to the amount of nitrogen removed in the *Vicia* crops in the year of the soil sampling, and assume that there had been only as much at the disposal of the plant as in the case of the *Trifolium* plot, it is obvious that the deficiency in the *Vicia* soils very nearly corresponds with the amount removed in the crop, which was about 100 lbs. Indeed, it may safely be concluded that most, if not the whole, of the nitrogen of the *Vicia* crops had been taken up as nitric acid.

But there had probably been more loss by drainage from the *Trifolium* plot without growth than from the *Vicia* plots with growth, and with, at the same time, much more upward passage and evaporation.

It must also be borne in mind, that the *Vicia* plots had, during the preceding 5 years, 1878 to 1882, yielded an average of more than 70 lbs. of nitrogen per acre, and in the immediately preceding year (1882), 146 lbs. Further, the amounts taken up by the plants each year must have been much greater than the amounts removed in the crops; for there must have been annually a large crop-residue, which would yield nitric acid for succeeding crops. Much of these large amounts of nitrogen must obviously have had some other source than the original surface soil, since it gained rather than lost under the treatment. If this source were not the atmosphere, but the subsoil, it must have been taken up, either as nitric acid, as some other product of the change of the organic nitrogen of the subsoil, or as organic nitrogen itself. Further, as the *Vicia* crops were large in the previous year, 1882, so also would their nitrogenous crop-residue be large, and contribute correspondingly large amounts of nitric acid for the crops of 1883. But the crops of 1883 were also large, and they, in their turn, would leave correspondingly large nitrogenous crop-residues; leaving a large proportion of the amount of nitrogen removed in the crops to be otherwise provided for than by previous residue.

Lastly in reference to these experiments, it is seen that at each of the 12 depths, down to 108 inches, the *Vicia* plots where there had been growth, contained less nitric-nitrogen than the *Trifolium repens* plot where there had been no growth. The difference is much the greatest in the first 18 inches, within which the *Vicia* throws out by far the larger amount of root; but it is very distinct below this point, and the supposition is that, under the influence of the growth of the *Vicia*, water had been brought up from below, and with it nitric acid. In fact, compared with the *Trifolium repens* plot, the mean for the two *Vicia* plots showed less water in the soil down to

108 inches, corresponding to between 6 and 7 inches of rain, or to between 600 and 700 tons of water per acre.

After this summary of previously published results we may now turn to the consideration of new results of the same kind.

2. *New Determinations of Nitric Acid in Soils and Subsoils.*

The plots experimented upon are in the same series, with the same previous history, as those already referred to. *Trifolium repens* was again selected as the weak and superficially rooting plant; *Melilotus leucantha* was taken as a deeper and stronger rooting one; and *Medicago sativa*, or lucerne, as a still deeper and still stronger rooting plant. Samples of soil were taken at the end of July and the beginning of August, 1885, from 2 places on each plot, and in each case, as before, to 12 depths of 9 inches each, equal to a total depth of 108 inches or 9 feet.

The following table (III.) shows the estimated yields of nitrogen per acre in the different crops during the experimental period from 1878 to 1885, the year of soil sampling, inclusive. The yields during the preliminary period have been already given in Table I.

TABLE III.—Estimated yield of Nitrogen per acre, in lbs., in wheat alternated with fallow, and in various leguminous crops, without nitrogenous manure.

	Unmanured.	Mineral manures only.		
	Fallow-wheat.	*Trifolium repens.*	*Melilotus leucantha.*	*Medicago sativa.*
	lbs.	lbs.	lbs.	lbs.
1878	29	0	53	Not sown
1879	Fallow	82	130	0
1880	24	0	36	28
1881	Fallow	8	60	28
1882	18	74	145	111
1883	Fallow	0	27	143
1884	29	0	56	337
1885	Fallow	97	58	233*
Total 8 years . .	100	261	565	880
Average annual .	12	33	71	(110)

Thus, the wheat plot was again fallow when sampled; the total yield of nitrogen in the crops in the 8 years was only 100 lbs. per acre, and the average annual yield little more than 12 lbs.

The *Trifolium repens* plots, after giving no crop in either 1883 or 1884, yielded produce containing nearly 100 lbs. of nitrogen in 1885, before the soil sampling; the total yield of nitrogen in the 8 years was 261 lbs., and the average annual yield 33 lbs.

* First and second crops only; a third crop, cut after the soil sampling, yielded 37 lbs. nitrogen.

The deeper rooting, and freer growing *Melilotus leucantha* gave more or less produce in each year of the eight, large crops in 1879 and 1882, a total yield of nitrogen over the eight years of 565 lbs. per acre, and an average annual yield of 71 lbs.

Lastly, the still deeper rooting, and still freer growing *Medicago sativa*, sown first in 1879, gave no crop in that year, only small crops in 1880 and 1881, and then rapidly increasing amounts, until the yield of nitrogen was estimated at 337 lbs. per acre in 1884, and at 233 lbs. in 1885 before the soil sampling, and 37 lbs. afterwards, making a total for that year of 270 lbs. The total yield of nitrogen in the 6 years, prior to the soil sampling, was 880 lbs.; giving an average over the 8 years of 110 lbs., or over the 6 years when there was any crop, of 147 lbs. of nitrogen per acre per annum.

Table IV. shows the amounts of nitrogen as nitric acid found in the soils and subsoils of the different plots—in all cases calculated into lbs. per acre.

TABLE IV.—Nitrogen as Nitric Acid per acre, lbs., in the soils and subsoils of some experimental plots, without nitrogenous manure, for more than 30 years. Hoosfield, Rothamsted. Samples collected July 29 to August 14, 1885.

Depths.	Unmanured.	Series I. Mineral manures only.			*Trifolium repens.* + or − Wheat land.	+ or − *Trifolium repens.*	
	Wheat-fallow land.	*Trifolium repens.* Plot 5.	*Melilotus leucantha.* Plot 5.	*Medicago sativa.* Plot 5.		*Melilotus leucantha.* Plot 5.	*Medicago sativa.* Plot 5.
inches.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1– 9	17·44	11·50	4·35	8·88	− 5·94	− 7·15	− 2·62
10– 18	3·67	1·38	1·40	1·11	− 2·29	+ 0·02	− 0·27
19– 27	2·76	0·90	2·12	0·78	− 1·86	+ 1·22	− 0·12
28– 36	2·16	1·86	2·94	0·81	− 0·30	+ 1·08	− 1·05
37– 45	1·68	7·08	5·22	0·99	+ 5·40	− 1·86	− 6·09
46– 54	1·47	11·31	6·21	0·93	+ 9·84	− 5·10	−10·38
55– 63	1·77	13·14	7·95	0·57	+11·37	− 5·19	−12·57
64– 72	1·83	12·63	10·08	0·81	+10·80	− 2·55	−11·82
73– 81	2·29	11·19	9·66	0·70	+ 8·90	− 1·53	−10·49
82– 90	2·01	10·70	9·16	0·61	+ 8·69	− 1·54	−10·09
91– 99	1·98	11·08	8·83	0·44	+ 9·10	− 2·25	−10·64
100–108	2·06	9·96	10·12	0·41	+ 7·90	+ 0·16	− 9·55
Total	41·12	102·73	78·04	17·04	+61·61	−24·69	−85·69
Summary and control.							
1– 9	17·44	11·50	4·35	8·88	− 5·94	− 7·15	− 2·62
10– 18	3·67	1·38	1·40	1·11	− 2·29	+ 0·02	− 0·27
Mixture of 19–108 inches	20·63	88·02	73·21	6·97	+67·39	−14·81	−81·05
Total	41·74	100·90	78·96	16·96	+59·16	−21·94	−83·94

The determinations of nitric acid in the soil extracts, the results of which are recorded in the table, as well as those given in Table II., were made in the Rothamsted Laboratory by Mr. D. A. Louis, by Schlœsing's method, as nitric oxide by the reaction with ferrous salts. For each of the twelve depths a mixture of the samples from the two holes was prepared, and in each of these mixtures duplicate determinations of nitric acid were made. As a control, determinations were also made in a mixture of the samples from the 10 lower depths, the third to the twelfth inclusive, and the results are given at the foot of the table.

The first point to remark is, that there was much less nitrogen as nitric acid in the *Trifolium repens* soil in 1885, after the removal of nearly 100 lbs. of nitrogen in the crops, than in 1883, when no crop had grown. The deficiency is the greatest in the 2 upper layers, but it extends to the fifth depth, amounting to that point, which represents the range of the direct or indirect action of the superficial roots of the plant, to about 61 lbs. Below the range of this action, however, there is even more nitrogen as nitric acid in 1885 than 1883; due doubtless in part to percolation from above during the two preceding seasons without growth, and possibly in part to percolation of the nitrifying organisms, and nitrification of the nitrogen of the subsoil.

Let us now turn to the results obtained on the *Melilotus leucantha* plot. As shown in Table III., it is estimated that, in 1882 as much as 145 lbs. of nitrogen was removed in the crop, and samples of soil taken that autumn, to the depth of 6 times 9 inches, or 54 inches in all, showed only 8·45 lbs. of nitrogen as nitric acid remaining, which was 17·8 lbs. less than was found to the same depth in the *Trifolium repens* plot which had yielded only 74 lbs., or only about half as much in the crop as the *Melilotus*.

After 1882, however, the produce of the *Melilotus* declined very much, and in 1885 the yield of nitrogen in the crop was estimated at only 58 lbs., against 97 lbs. estimated to have been removed in the *Trifolium repens* crop. Under these circumstances the *Trifolium repens* soil shows even rather less nitric acid than the *Melilotus* soil, in the second, third, and fourth depths, which comprise the chief range of action of the *Trifolium repens* roots. At every depth below the fourth, however (except the 12th, where the difference is very small), there is notably less nitrogen as nitric acid in the *Melilotus* than in the *Trifolium repens* soil, the *Melilotus* having yielded so much more in its crops in the preceding years than the *Trifolium repens*. To the total depth of 108 inches there was 24·69 lbs. less nitric nitrogen remaining in the *Melilotus* than in the *Trifolium repens* soil.

Admittedly we cannot know what was the stock of nitric nitrogen in either soil at the commencement of the growth of the season. But as during the 8 years 565 lbs. of nitrogen were removed in the *Melilotus* crops, against only 261 lbs. in the *Trifolium repens*, or more than twice as much in the *Melilotus* as in the *Trifolium repens*, it may be supposed that the *Melilotus* would both leave more nitrogenous crop-residue

for nitrification, and with its deeper roots, would each year the more exhaust the nitric nitrogen especially of the lower layers. Hence, notwithstanding the much lower yield of nitrogen in the *Melilotus* than in the *Trifolium repens* crop in 1885, the lower layers of the *Melilotus* soil contained less nitric acid than those of the *Trifolium repens* soil. There can, indeed, be no doubt, that the *Melilotus* derived at any rate much of its nitrogen from nitric acid, either within the actual range of its roots, or within the range of their action in causing the passage upwards of water with its dissolved contents. Still, the figures show, that with the comparatively limited growth in the recent years, there remained per acre about 56 lbs. of nitrogen as nitric acid in the 6 lower depths of the *Melilotus* soil.

But by far the most striking results in the Table are those relating to the *Medicago sativa* (lucerne) soil, and to the comparison between the amounts of nitric nitrogen in the soil of the shallow rooting and weakly growing *Trifolium repens* and those in the soil of the very deep and strong rooted, and very free growing lucerne.

Table III. shows that the estimated yields of nitrogen per acre in the lucerne were in the 6 years, 1880–1885, respectively as follows:—28 lbs., 28 lbs., 111 lbs., 143 lbs., 337 lbs., and 233 lbs. That is to say, with the increasing root range, and consequently increased command of the stores of the soil and subsoil, the yield of nitrogen in the crop increased from 28 lbs. in the first and second years, to 337 in the fifth year; declining, however, somewhat in the sixth year, 1885, and it did so still further in 1886.

It is seen that under these circumstances of very large yields of nitrogen in the crops, there is, at every one of the twelve depths, less, and at most very much less nitrogen as nitric acid remaining in the soil than where so much less nitrogen had been removed in the *Trifolium repens* crops. The difference is distinct even in the upper layers, but it is very striking in the lower depths. Thus, there is, on the average, not one-twelfth as much nitric nitrogen in the lower ten depths of the deep rooting and high nitrogen-yielding *Medicago sativa* soil, as in those of the shallow rooting and comparatively low nitrogen-yielding *Trifolium repens* soil. Indeed, the nitric acid is nearly exhausted in the deep rooting *Medicago sativa* plot; there remaining, to the total depth of 9 feet, only about 17 lbs. of nitric nitrogen against more than 100 lbs. to the same depth in the *Trifolium repens* soil. The total deficiency of nitric nitrogen in the *Medicago* as compared with the *Trifolium repens* soil, is seen to be 85·69 lbs. according to one set of determinations, and 83·94 lbs. according to the other.

As already said, we cannot know what was the stock of nitric nitrogen in the soil at the commencement of the growth of the season, or the amount formed during the growing period. But with so much more *Medicago* growth for several previous years, it seems reasonable to assume that there would be much more nitrogenous crop-residue for nitrification than in the case of the *Trifolium repens* plot.

But even supposing, for the sake of illustration, that each year's growth would leave crop-residue yielding an amount of nitrogen as nitric acid for the next crop, or succeeding crops, approximately equal to the amount which had been removed in the

crop, the increasing amounts of nitrogen yielded in the crops from year to year could not be so accounted for; and there would remain the amount of nitrogen in the crop-residue itself, still to be provided in addition. In fact, assuming the proportion of nitrogen in the crop-residue to that in the removed crop to be as supposed in the above illustration, nearly 700 lbs. of nitrogen would have been required for the *Medicago* crop and crop-residue of 1884; or if we assume the nitrogen in the residue to be only half that in the crop, about 500 lbs. would have been required. Doubtless, however, some of the nitrogenous crop-residue would accumulate from year to year.

The results can leave no doubt that the *Trifolium repens*, the *Melilotus leucantha*, and the *Medicago sativa*, have each taken up much nitrogen from nitric acid within the soil. But, at any rate so far as the *Medicago* is concerned, there is nothing in the figures to justify the conclusion that the whole of its nitrogen can have been so derived. It is obvious that if nitric acid were the source of the whole there must have been a great deal formed by the nitrification of the nitrogen of the subsoil. The alternative is—provided the atmosphere be not the source—that the deep-rooted plant takes up nitrogen from the subsoil in some other way.

3. *Percentage of Nitrogen in the Surface Soils of the Experimental Plots.*

It has been stated in general terms that, although much more nitrogen had been removed from the leguminous crop soils than from the fallow-wheat land, for nearly 30 years prior to the commencement of the experiments with various leguminous plants in 1878, yet the leguminous crop surface soil remained rather richer in nitrogen than the fallow-wheat soil. It has also been stated that, during the subsequent years of experiment with the various leguminous plants, the surface soil had gained rather than lost nitrogen. It will be well to give a summary of the actual experimental results relating to these points.

In the first place it is to be borne in mind, as Table I. (p. 5) shows, that whilst over the 27 years 1851–1877 inclusive, only about 17 lbs. of nitrogen were removed per acre per annum in the wheat grown in alternation with fallow, there was, over the 29 years, 1849–1877 inclusive, an average of about 32 lbs., or nearly twice as much, removed from the adjoining clover plots.

During the years 1878, 1879, and 1880, the yield of nitrogen in the wheat was about the same as the average of the preceding 27 years; whilst in most of the leguminous crops the yield was more than over the preceding 29 years. In the autumn of 1880 all the plots were ploughed up, and at the end of March, 1881, before resowing, soil samples were taken from five places on the leguminous crop-land, and also from five on the portion of the wheat land which was then fallow. The samples were, in each case, taken to the depth of 3 times 9 inches, or 27 inches in all.

It has already been stated that no nitrogenous manure had been applied to either the fallow-wheat, or the leguminous crop land, for more than 30 years; but that to

the leguminous crop land different mineral manures had occasionally been applied.

They were as follows :—

Plot 2. Superphosphate of lime.

Plot 3. Sulphate of potash.

Plot 4. Sulphate of potash and superphosphate.

Plot 5. Salts of potash, soda, lime, and magnesia.

Plot 6. As 5, with superphosphate in addition.

Soil samples were taken from each of the 5 plots, and also from 5 places directly opposite to them on the immediately adjoining fallow-wheat land. The following table (V.) shows the percentage of nitrogen in the fine sifted surface soil, 9 inches in depth, as dried at 100° C., in the sample from each plot. It also shows the mean for the five plots, the mean of determinations made on mixtures from the five plots, and the general means of these two sets of determinations. It should be added that each figure given is the mean of three or more determinations made by the soda-lime method.

TABLE V.—Nitrogen per cent. in soil samples collected March, 1881.

Nitrogen per cent. in fine surface soil, 9 inches deep. Calculated on the soil as dried at 100° C.		
	Leguminous crop soils.	Fallow-wheat soil.
	Per cent.	Per cent.
Plot 2	0·1064	0·0938
„ 3	0·1036	0·0930
„ 4	0·0950	0·0931
„ 5	0·1100	0·0957
„ 6	0·1156	0·1007
Means	0·1061	0·0953
On mixture	0·1055	0·0984
General means	0·1058	0·0969

There can be no doubt that both the leguminous crop and the fallow-wheat surface soils had lost nitrogen during the preceding 30 years or more ; but whilst in 1881 the surface soil of the plots which had grown many leguminous crops showed an average of 0·1058 per cent. of nitrogen, that of the plot which had grown wheat in alternation with fallow, and yielded over so many years only about half as much in crops, contained only 0·0969 per cent. It may be thought that the difference is not great ; but a glance at the details which give these means can leave no doubt that it is real ; nor can there be any doubt that it is characteristic also. The further results will afford confirmation of this.

The next point to consider is, whether the continued growth of the various leguminous crops has reduced or increased the stock of nitrogen in the surface soils The foregoing Table shows that in 1881 samples were taken from each of the five differently mineral-manured plots, but in 1882, 1883, and 1885, when samples were taken to considerable depths for the determination of the nitric acid, either plot 4, plot 5, or plot 6 was always selected, as on them the growth was better than on either plot 2 or plot 3.

The following Table (VI.) summarises the percentages of nitrogen in the surface soils (9 inches deep) of the fallow-wheat land, of the *Trifolium repens* plots, of the *Vicia sativa* plots, of the *Melilotus leucantha* plots, and of the *Medicago sativa* plots, in the years as indicated. The figures are as before in all cases the means of 2, 3, or more determinations on each sample. In each case the results given in the first line are the means of determinations made on the individual samples taken from different places on the plot, those in the second line are the means of the determinations made on mixtures of the individual samples, and the general means given in the third line are the means of the results on the individual and on the mixed samples, taken together. It may be further explained that the wheat-fallow samples were taken in 1883 from 4, and in 1885 from 3 places on the plot. The *Trifolium repens* samples were taken in 1882 from 2 places on plot 6, in 1883 from 2 places on plot 4, and in 1885 from 2 places on plot 5. The *Vicia sativa* soils were taken in 1883 from 2 places on plot 4, and from 2 on plot 6. The *Melilotus leucantha* soils were taken in 1882 from 2 places on plot 5, and from 2 on plot 6, and in 1885 from 2 on plot 5. Lastly, the *Medicago sativa* soils were taken in 1885 from 2 places on plot 5. The determinations were, as before, made by the soda-lime method.

TABLE VI.—Percentages of Nitrogen in the fine sifted surface soils (9 inches deep), reckoned as dry at 100° C., at different periods. Samples collected July 26–31, 1882; July 17–26, 1883; July 29–August 14, 1885.

	1882.	1883.	1885.	Mean
Wheat-fallow land.				
	Per cent.	Per cent.	Per cent.	Per cent.
Means on individual samples	..	0·1044	0·1006	0·1025
Means on mixtures of individual samples	..	0·1026	0·1035	0·1031
General means	..	0·1035	0·1021	0·1028
***Trifolium repens* land.**				
Means on individual samples	0·1149	0·1131	0·1269	0·1183
Means on mixtures of individual samples	0·1131	0·1125	0·1268	0·1175
General means	0·1140	0·1128	0·1269	0·1179
***Vicia sativa* land.**				
Mean on individual samples	..	0·1203	..	0·1203
Mean on mixture of individual samples	..	0·1178	..	0·1178
General mean	..	0·1191	..	0·1191
***Melilotus leucantha* land.**				
Means on individual samples	0·1095	..	0·1122	0·1109
Means on mixtures of individual samples	0·1123	..	0·1179	0·1151
General means	0·1109	..	0·1151	0·1130
***Medicago sativa* land.**				
Mean on individual samples	..	..	0·1214	0·1214
Mean on mixture of individual samples	..	..	0·1224	0·1224
General mean	..	..	0·1219	0·1219

It is seen that even the fallow-wheat soils show slightly higher percentages of nitrogen in the autumns of 1883 and 1885 than in March, 1881; but the later samples were all taken from the end of the field, the samples from which showed somewhat higher percentages than the others even in 1881; and, as the figures show,

the increase was in most cases much greater on the various leguminous plots. Thus, comparing the final means in the foregoing Table with those in Table V. (p. 14), the percentage in the wheat-fallow soil is 0·1028 against 0·0969 in 1881 ; in the *Trifolium repens* soil it is 0·1179, in the *Vicia sativa* soil 0·1191, in the *Melilotus leucantha* soil 0·1130, and in the *Medicago sativa* soil 0·1219, against a general mean of 0·1058 in 1881.

It should be stated that before taking the samples all above-ground growth is carefully cut off by scissors and removed ; and that in the preparation of the soil-samples for analysis, all roots, indeed all visible vegetable débris, is carefully picked out ; so that the results only include the nitrogen of that part of the crop-residue which has become thoroughly disintegrated, and may be considered as a proper constituent of the surface mould. It may be further stated that the separated residue from the leguminous crop soils contained more nitrogen than that from the wheat-fallow soil.

Going a little more into detail, it is seen that the *Trifolium repens* soil shows a mean of 0·1140 per cent. nitrogen in 1882, of 0·1128 per cent. in 1883, and of 0·1269 per cent. in 1885. That is to say, the lowest percentage is in 1883 when there had been no growth, when there had been a whole season for the disintegration and nitrification of the residue of the previous year, and when 146 lbs. of nitrogen as nitric acid were found to the depth of 108 inches, and more than four-fifths of it below the surface soil. On the other hand, the percentage is the highest in 1885, when nearly 100 lbs. of nitrogen had recently been removed in the crop, and the crop-residue would be comparatively large.

In the *Melilotus leucantha* soil somewhat more nitrogen was found in 1885, which was the eighth year of continuous crop, than in 1882, which was only the fifth season, but which yielded more than twice the amount of nitrogen in the crop, and left considerably more visible and separated residue in the surface soil.

Lastly, of the *Medicago sativa* soils, we have samples only in the sixth year of the growth, which had rapidly increased to an enormous amount in the fifth year, 1884, and yielded very large, though somewhat less, produce in 1885. Under these circumstances the mean percentage of nitrogen in the surface soil is 0·1219, or higher than in the case of any other plant or year, excepting in the *Trifolium repens* soil of the same year. This is the case notwithstanding that more visible crop-residue had been separated from the *Medicago sativa* soil samples than from any of the others ; and in fact about three times as much as from the *Trifolium repens* soils of the same year. Indeed, it was estimated that the separated residue from the *Medicago sativa* soil-samples, the nitrogen in which was determined, represented a removal of about 100 lbs. of nitrogen per acre.

Without relying on the exact figures as representing exact gains or losses of nitrogen by the surface soils, we think it will be granted that the results are too consistent to leave any doubt that by the growth of the leguminous crops the surface soils had gained nitrogen, and that this gain bore some relation to the amount of growth and removal in the crops.

If then the surface soils have gained in nitrogen, it is obvious that they have not been the primary source of the nitrogen taken up by the plants. It must have come either from above or from below the surface soil—from the atmosphere or from the subsoil. The evidence does indeed point to the fact, that much nitric acid results from nitrification of nitrogen accumulated within the surface soil. But as this nitrogen either increases or does not diminish, much of the nitric acid produced must come from some other source than what may be called the original stock of nitrogen of the surface soil itself. Much doubtless comes from the nitrogen of crop-residue, itself derived from the atmosphere or from the subsoil.

Before discussing this subject further it will be well to call attention to another remarkable experiment, showing the amount of nitrogen that may be taken up by one leguminous plant growing on land where another had to a great extent failed.

4. *Experiments on the growth of Red Clover on Bean-exhausted Land.*

The results in question were obtained in a field in which beans had been grown almost continuously for 32 years, but had considerably declined in yield, as the following Table will show :—

TABLE VII.—Quantities of Nitrogen removed per acre per annum, in lbs., in bean-crops, over four periods of 8 years each. Geescroft Field, Rothamsted.

	8 years, 1847–1854.	8 years, 1855–1862.	8 years, 1863–1870.	8 years, 1871–1878.	Average 32 years, 1847–1878.
	lbs.	lbs.	lbs.	lbs.	lbs.
1. Without manure	48·41	25·26	9·12	16·36	24·79
2. With mineral manure containing potash	60·19*	34·25	23·46	26·66	35·36†
3. With mineral manure and nitrogen . .	68·94	36·87	35·05	28·69	42·39

The two upper lines show the amounts of nitrogen removed per acre per annum in the bean crops without any supply of nitrogen by manure. It will be seen that over each period of 8 years, the plot receiving a mineral manure containing potash yielded considerably more nitrogen than the one without any manure. In both cases, however, there was considerable decline from the first period to the last. Further, whilst over the 32 years the unmanured plot yielded an average of 24·8 lbs. of nitrogen per acre per annum, that with the potash manure yielded 35·4 lbs., or nearly one-half more, though without any supply of nitrogen from without. In the third experiment, where besides the potash manure some nitrogen was applied, in the early years as ammonium

* Average of 7 years only, results not available for 1849.

† Average 31 years only.

salts and in the later as sodium nitrate, there is some, but comparatively little increase in the amount of nitrogen in the crop.

In connection with the fact of the gradual decline in yield, it should be explained that owing to failure of the beans there was—in the second period one year of fallow, and one year of wheat; in the third period, one year of fallow; whilst in the fourth period, the first crop failed, and the land was left fallow during the second, third, and sixth seasons. The yields of nitrogen are, however, in each case, averaged for the period of 8 years.

After the 32nd year, 1878, the land was left fallow for between four and five years. Under these circumstances, as will be seen presently, the stock of total nitrogen in the surface soil had become very low, direct determinations of the nitrogen as nitric acid showed that the already existing amount of nitric-nitrogen down to the depth of 72 inches, was extremely small, whilst after several years of fallow there would be a minimum amount of crop-residue remaining for nitrification.

On this land, exhausted for one leguminous crop, barley and clover were sown in the spring of 1883. The clover grew very luxuriantly from the first, much interfering with the growth of the barley.

In our paper in the 'Transactions of the Chemical Society,' for June, 1885, we gave the amount of nitrogen as nitric acid found to the depth of 72 inches on the plot without manure, in that with the mineral manure alone, in that with the mineral and nitrogenous manure, and in that with farm-yard manure. We further estimated that the barley and clover crops would probably remove more than 200 lbs of nitrogen per acre. The amounts have, however, since been determined, and they are as follows:—

TABLE VIII.—Nitrogen removed per acre in the barley and clover crops.

Previous condition of manuring.	1883. Barley and clover.	1884. Clover.	1885. Clover.	Total.
	lbs.	lbs.	lbs.	lbs.
Without manure	45·0	183·2	52·7	280·9
Mineral manure and some nitrogen . .	57·2	193·1	79·9	330·2
Mineral manure only	59·3	206·4	81·6	347·3

It should be stated that the plots, the yield of nitrogen of which is here given, do not exactly correspond with those as given in the preceding Table, some of the crops being taken together where no difference in the produce was observable. Thus half the plot represented as without manure has been unmanured from the commencement, that is, for nearly 40 years, the other half having received small quantities of nitrogen to 1878 inclusive, but has since been entirely unmanured. Again, the results given in the second line relate to the produce on the plot with the purely mineral manure containing potash, given in Table VII. as No. 2, together with that of the plot No. 3 to

which some ammonium salts, or nitrate had, up to 1878, been applied, but which has received no manure since. The results given in the third line of the above Table (VIII.), relate, however, to a plot which has not received any nitrogenous manure from the commencement, but was not brought into experiment until five years later than the other plots.

Here then, in a field where beans had been grown for many years, had frequently yielded only small crops, and sometimes failed, and the land had then been left fallow for several years, where the surface soil had become very poor in total nitrogen, where both surface and subsoil were very poor in ready formed nitric acid, and where there was a minimum amount of crop-residue near the surface for decomposition and nitrification, there were grown very large crops of red clover containing very large amounts of nitrogen. On a plot where a purely mineral manure, containing potash, had been applied for 27 years up to 1878, but no manure whatever since, 347 lbs. of nitrogen were gathered, almost wholly by the clover. On a plot, on half of which the mineral manure only, and on the other half the same mineral manure with some ammonium salts or nitrate had been applied up to 1878, but nothing since, 330 lbs. of nitrogen were removed in the crops. Lastly, where on half the plot there had been no manure whatever for nearly 40 years, and on the other half ammonium salts or nitrate to 1878, but nothing since, 281 lbs. of nitrogen were yielded in the crops.

It may be said, therefore, that about 300 lbs. of nitrogen had been gathered by the clover growing on a soil upon which beans had yielded smaller and smaller crops, and, in fact, had eventually practically failed, and which was very poor both in total nitrogen near the surface, very poor in ready formed nitric acid to a considerable depth, and very poor in nitrogenous crop-residue for nitrification. If therefore the clover had taken up its nitrogen either wholly or mainly as nitric acid, the supply could not be due to recent crop-residue.

Not only was so much nitrogen removed in the crops, but the surface soil became determinably richer in nitrogen, as the following results will show. The plots are the same as those to which Table VII. refers; and the determinations are those made in samples of surface soil collected in April, 1883, before the sowing of the barley and clover, and in November, 1885, after the removal of the crops.

TABLE IX.—Nitrogen per cent., and per acre, in the surface soils, before and after the growth of the barley and clover. Geescroft Field, Rothamsted.

	Nitrogen in sifted dry soil.				
	Per cent.		Per acre.		
	1883.	1885.	1883.	1885.	1885 + or − 1883.
	p. c.	p. c.	lbs.	lbs.	lbs.
1. Without manure	0·0993	0·1083	2441	2662	+ 221
2. With mineral manure containing potash	0·1087	0·1149	2672	2824	+ 152
3. With mineral manure and nitrogen	0·1163	0·1225	2859	3011	+ 152

Without assuming that the figures represent accurately the amounts of nitrogen accumulated per acre, it cannot be doubted that the surface soils had become considerably richer. If, for the sake of illustration, we assume that 300 lbs. of nitrogen were removed per acre in the crops, and 200 lbs. were accumulated in the surface soil, we have 500 lbs. to account for as gathered by the crops, and chiefly by the clover, within about 2 years.

In our former paper, when we assumed that perhaps 200 lbs. would be removed in the crops, we admitted that there was in the experimental results no conclusive evidence as to the source of so large an amount of nitrogen, but that it must obviously have been derived either from the atmosphere or from the subsoil; and assuming it to be the subsoil rather than the atmosphere, the question arose whether it was taken up as nitric acid, as ammonia, or as organic nitrogen? It was pointed out that as yet no direct proof existed that green-leaved plants did take up the organic nitrogen of the soil as such; and that although there was more evidence from analogy in favour of a nitric acid source than of any other, proof was equally wanting to establish the conclusion that so much nitrogen had been available as nitric acid. The much larger amounts now known to have been gathered by the clover crop, of course renders this explanation still less adequate.

On a review of the whole of the results that have been adduced, it cannot be doubted that nitric acid is an important source of the nitrogen of the Leguminosæ. Indeed, so far as existing experimental evidence goes, that relating to nitric acid carries us quantitatively further than any other line of explanation. But it is obviously quite inadequate to account for the facts of growth, either in the case of the *Medicago sativa* grown on the clover-exhausted land, or in that of the clover on the bean-exhausted land. There is, in fact, nothing in the results relating to the clover experiment to justify the conclusion that there had been such a large production of nitric acid in the subsoil, due to the increased development of the nitrifying organisms under the influence of the leguminous growth and crop-residue, and their

distribution, favoured by the action of the roots, and the increased activity in the interchange of moisture and air which must take place under such circumstances. Nor is it explicable how such large quantities of nitric acid could have been produced as would be required for the rapidly increasing growth of the *Medicago sativa*, and for the large amounts of nitrogen in it, if nitric acid had been the exclusive or even the main source of supply.

5. *Experiments on the Nitrification of the Nitrogen of Subsoils.*

In our paper in the 'Transactions of the Chemical Society' already referred to, we showed, in the case of some prairie land subsoils, that their nitrogen was susceptible of nitrification, and that when, after repeated extraction, the action became very feeble, or ceased, it was renewed on the soils being seeded by 0·1 gram of rich garden mould, which would contain nitrifying organisms. Considering, however, that from the circumstances of the collection and the transmission of those samples, the entire exclusion of comparatively recent organic residue from the upper layers was uncertain, it was decided to experiment in a similar way with some of the Rothamsted raw clay subsoils. Those selected were :—

1. A mixture of samples from the third to the twelfth depths of 9 inches each, that is representing the layer of 90 inches thick from 19 to 108 inches deep, from each of 3 out of the 4 holes from which samples were taken on the wheat-fallow land from July 17–26, 1883.

2. Similar mixtures from the third to the twelfth depths, from the samples taken from July 17–26, 1883, from the *Trifolium repens*, and from each of the two *Vicia sativa* plots, respectively.

3. From holes opened specially for subsoil samples only, one on the wheat-fallow land, on May 7, 1886, and one on April 16, 1886, in a field where red clover had been sown with barley in rotation in the previous spring, but from which no crop had yet been taken.

The first column of the next Table (X.) shows, for each of these samples, the percentage of total nitrogen calculated on the dry sifted soil, as determined by the soda-lime method; and the second column shows the quantity of already existing nitric-nitrogen per million of fine dry soil in each case. The other columns show the amounts of nitric-nitrogen per million dry soil, as determined by Schlœsing's method, in watery extracts made by the aid of the water pump, after successive periods of exposure under suitable conditions as to temperature and moisture. In the case of the wheat-fallow and leguminous crop subsoils, the results relating to which are given in the upper two divisions of the Table, they were each seeded by the addition of 0·1 gram of rich garden soil after the first period of exposure and subsequent extraction, and again by the addition of 0·2 gram after the third period and extraction. These experiments, with the exception of the determinations of the total nitrogen, were made in the Rothamsted Laboratory by Mr. D. A. Louis.

In the case of the wheat-fallow, and rotation clover land samples, to which the bottom division of the Table refers, there was a seeding with 0·2 gram of the garden soil after the extraction of the already existing nitric nitrogen, and before the first period of exposure; and there was again a similar seeding of the "seeded" lots, after the third period of exposure in the case of the wheat-fallow subsoil, and after the fourth period in that of the rotation clover land subsoil. There was also added, in the case of the wheat-fallow subsoil after the third, and in that of the clover subsoil after the fourth period, 0·8 gram of a mixture of 1 part potassium phosphate, 1 part magnesium sulphate, and 5 parts calcium carbonate; the mixture containing as impurity 0·000616 gram nitrogen per gram.

TABLE X.—Results showing the amount of the Nitrification of the nitrogen of subsoils.

	Nitrogen in fine dry soil.	Nitrogen as nitric acid per million fine dry soil.														Total.
		Original.	Periods of exposure.													
			1st.			2nd.			3rd.			4th.			5th †	
Wheat-fallow subsoils, collected July 17–26, 1883. Mixture of 3rd–12th depths.																
	Per cent.		Days.			Days.			Days.			Days.			Days.	
Hole 1	·0492	0·77	28	·106		28	·109		28	·237		28	·247	246	·084	358	·783
„ 2	·0562	0·48	28	·071		28	·208		28	·043		28	·162	246	·225	358	·709
„ 4	·0484	0·54	28	·127		28	·166		28	·066		28	·299	246	·224	358	·882
Means	·0513	0·60	28	·101		28	·161		28	·115		28	·236	246	·178	358	·791
Leguminous crop subsoils, collected July 17–26, 1883. Mixture of 3rd–12th depths.																
Trifolium repens. Plot 4	·0592	2·50	28	·132		28	·083		28	·156		28	·303	246	·338	358	1·012
Vicia sativa. „ 4	·0508	1·63	28	·145		28	·015*		28	·185		28	·379	246	·375	358	1·099
Vicia sativa. „ 6	·0360	1·40	28	·108		28	·079		28	·176		28	·276	246	·421	358	1·060
Means	·0487	1·84	28	·128		28	·059		28	·172		28	·319	246	·378	358	1·056
Wheat-fallow samples collected May 7, 1886, at a depth of 5 feet. Clover land samples collected April 16, 1886, at 7 feet 4 inches deep.																
Unseeded { Wheat-fallow	·0768	0·720	28	·053		35	·052		49	·107		129†	[·312]‡			241	·524
Unseeded { Clover land	·0772	0·618	28	·077		35	·060		28	·134		30	·177	139	[·358]	260	·806
Means	·0770	0·669	28	·065		35	·056		39	·121		79	·245				
Seeded { Wheat-fallow	·0768	0·714	28	·140		35	·081		49	·118		129†	[·358]			241	·697
Seeded { Clover land	·0772	0·626	28	·262		35	·103		28	·437		30	·192	139	[·580]	260	1·574
Means	·0770	0·670	28	·201		35	·092		39	·278		79	·275				

Referring to the results, it is in the first place to be observed that there is very considerable variation in the percentage of total nitrogen in the different subsoils.

* Probably too low.

† The number of days represents the periods of exposure, the periods of activity are uncertain.

‡ The figures given between brackets thus [ ] show the results obtained after the addition of the mineral mixture described in the text.

Indeed, so variable is the amount of nitrogen in samples of our Rothamsted subsoils taken on one and the same plot, dependent on the varying proportions of clay, sand, gravel, chalk, &c., that as we have fully illustrated in former papers, no estimates of the difference in the amounts of total nitrogen, either in the subsoil of the same plot at different periods, or in the subsoils of plots differently manured, or differently cropped, can be relied upon.

Again, as the amount of the "original" or already existing nitric-nitrogen is, as has been very fully shown, greatly dependent on the description, and on the amount of crop that has been grown, and other circumstances, it is not to be expected that it would bear any direct relation to the richness or poverty of the subsoil in total nitrogen.

Referring to the amounts of nitric-nitrogen formed in the different subsoils, and within the different periods of exposure, there is, as is to be expected in the case of an action depending on the development and activity of an organism of the habits, requirements, and mode of action of which we know but little, considerable irregularity, both from period to period with the same sample, and within each period with the different samples.

Confining attention in the first place to the results relating to the wheat-fallow, and the leguminous crop subsoils, recorded in the upper two divisions of the Table, it will be seen that the first, second, third, and fourth periods of exposure each comprised 28 days; whilst the fifth period extended over 246 days, or about 35 weeks, during a considerable portion of which, however, the soils had doubtless become too dry for activity and nitrification. It is probable that a period of 28 days is too short to insure the active development of the organisms, and consequent energetic nitrification. Then again, the extraction of the soils by water under pressure must, it is to be supposed, remove some of the organisms, instead of allowing of their natural multiplication. Comparison of the results from period to period must, therefore, be made with some reservation.

But apart from any irregularities in the case of individual samples, or at individual periods, if we compare for each period the mean results for the three wheat-fallow samples, with the means for the leguminous crop subsoils, it is seen that during four of the five periods, the leguminous crop subsoils show considerably more nitrification than the wheat-fallow ones; and whilst over the total period the wheat-fallow subsoils show an average of 0·791 nitrogen nitrified per million of soil, the leguminous crop subsoils show 1·056 per million.

Again, the figures in the bottom division of the Table, relating to the wheat-fallow and the rotation clover land samples, collected in the spring of 1886, show in all cases comparable as to length of period, more nitrification in the clover land than in the wheat-fallow land subsoil; and this is so both with the unseeded and the seeded samples.

Thus, then, these results with the raw, and mostly clay, Rothamsted subsoils, containing not more than 6 or 8 parts carbon to one of nitrogen, confirm those

previously obtained with the prairie subsoils containing much higher proportion of carbon, in showing that their nitrogen is susceptible to nitrification, provided the organisms, and other essential conditions, are not wanting. These new results also consistently show that there is more active nitrification in the leguminous than in the gramineous crop subsoils. This it must be supposed, is partly due to more active development, and greater distribution, of the organisms themselves, under the influence of the leguminous growth, with its excretions and residue, and partly to the greater actual amount of such easily changeable matters.

The results are also confirmed by those of experiments made in the Rothamsted Laboratory by Mr. WARINGTON, for the most part on quite distinct lines. Thus, in most cases, instead of determining the amount of nitrification taking place in the different subsoils when exposed under suitable conditions, he introduced a portion of the subsoil into a sterilised nitrogenous liquid, and determined whether nitrification took place; the result being taken to show whether or not the organisms were present in the subsoil. In the first experiments, the samples were taken with precautions to avoid any contamination by roots or other organic matter, and the conditions of the sterilised liquids were such as the experience of the time indicated as favourable for nitrification. Upon these results he says ('Chem. Soc. Trans.,' 1884, p. 645): "I am disposed to conclude that in our clay soils the nitrifying organism is not uniformly distributed much below 9 inches from the surface. On much slighter grounds, it may perhaps, be assumed, that the organism is sparsely distributed down to 18 inches, or possibly somewhat further. At depths from 2 feet to 8 feet, there is no trustworthy evidence to show that the clay contains the nitrifying organism. It is however probable that the organism may occur in the natural channels which penetrate the subsoil at a greater depth than in the solid clay."

Subsequently ('Chem. Soc. Trans.,' 1887, p. 118) he experimented with a greater variety of subsoils, taking samples from the wheat-fallow, the *Trifolium repens*, the *Melilotus leucantha*, and the *Medicago sativa* subsoils, when these were exposed for the collection of the samples for the various experiments, our own results relating to which we have given in some detail. Further, some of the samples were now taken in the immediate neighbourhood of lucerne roots, and gypsum was added to the sterilised liquids.

Among the 69 trials made in this new series of experiments, there was no failure to produce nitrification by samples down to 2 feet; there was only one failure out of 11 trials down to 3 feet; but below 3 feet, the failures were more numerous. Taken at 6 feet about half the samples induced nitrification. The order of priority of nitrification diminished from the upper to the lower depths; indicating more sparse occurrence, and more feeble power of development and action.

Examination of the results shows, however, that quite consistently with those which we have described, there was notably more active nitrification with the leguminous than with the gramineous crop subsoils. Thus, compared with the results yielded by

the wheat-fallow subsoils, those by the white clover subsoils were more marked; but this was especially the case with the lucerne plot subsoils, of which more samples, and those from a greater depth, induced nitrification. The same is also observable on a comparison of the results obtained by the samples from the wheat-fallow plot, with those from the rotation red clover plot.

It is then established that the nitrogenous matters of raw clay subsoils are susceptible of nitrification, if the organisms, with the other necessary conditions, are present. It is further indicated, not only that the action is more marked under the influence of leguminous than of gramineous growth and crop-residue, but that the organisms become distributed to a considerable depth even in raw clay subsoils, especially where deep-rooted and free-growing Leguminosæ have grown.

The next question is, how far, in a quantitative sense, do the results aid us in explaining the source of the large amounts of nitrogen taken up by some leguminous crops—as for instance in the case of the *Medicago sativa* grown on the clover-exhausted land, and of the red clover grown on the bean-exhausted land.

In the case of the three leguminous crop subsoils there was, over the total period, only about 1 part of nitrogen nitrified per million of soil; and as the subsoil to the depth experimented on would weigh about 30 million lbs. per acre, the amount of nitrification supposed would represent only about 30 lbs. per acre. Obviously, the conditions of nitrification in which the samples are exposed in the laboratory are very different from those of the subsoil *in situ*. Thus, whilst in the case of the samples in the laboratory, the conditions as to temperature and aëration would be the more favourable, the successive extractions by water under pressure would be liable to remove, not only the mineral matters essential for the development of the organisms, and for the production of nitric acid, but the organisms themselves, whereas in the case of the natural subsoil the tendency would be to multiplication.

Compared with the small amount of nitrification of the nitrogen of the raw clay subsoils shown in the foregoing experiments, in which some of the conditions were more and others less favourable than in the natural subsoil, the following results obtained by Mr. Warington ('Chem. Soc. Trans.,' 1887, pp. 127–9), show how very large may be the amount of nitrification of the nitrogen of such subsoils under more favourable circumstances than those of their natural condition. Thus, he mixed raw clay subsoil with an equal weight of coarsely powdered flint, seeded the mixture with rich garden soil, moistened it, and placed it in a vessel allowing for free access of washed air. Under these conditions he found, when no mineral food was added, in one case 2·4, and in another 3·0 per cent. of the total nitrogen of the subsoil nitrified; and when mineral food was added, he found in one case 4 per cent., and in another 3·6 per cent. of the total nitrogen was nitrified.

Indeed, the greatest difficulty in the way of the supposition that much nitrogen is available to plants by the nitrification of the nitrogen of the subsoil, is, in fact, the want of sufficient aëration. Independently of the greater or less porosity of the sub-

soil itself, and of the channels formed by worms, it is obvious that wherever the roots go, water and its contents can follow; and that, with deep-rooted plants and free growth, there will be active movement of water, and there must be of air also, in the lower layers of the soil. In our former paper we called attention to the fact that in the experiments in 1882, with the greater growth of the *Melilotus*, there remained in the soil less water than in that of the *Trifolium repens* soil, corresponding down to a depth of 54 inches, to a loss of 540 tons per acre, or nearly $5\frac{1}{2}$ inches of rain; and again, in 1883, the *Vicia sativa* soil showed down to 108 inches, less water than the *Trifolium repens* soil, in amount corresponding to between 600 and 700 tons per acre, or to between 6 and 7 inches of rain. Obviously too, the still deeper rooting, and still freer growing *Medicago sativa* would remove still more water.

Although much experiment and much calculation have been devoted by several investigators to the estimation of the degree of aëration of soils and subsoils of different character, the data at command do not justify any very definite conclusions on the subject. The results seem to indicate a probable range of aëration from about 30 to over 50 per cent. of the volume of the soil. But these estimates do not take into account the varying amounts of water in the soil or subsoil. In the case of the subsoils referred to in this paper, each layer of 9 inches in depth retained from about 2 to nearly 4 inches of water, the amount varying very much according to the nature of the subsoil, and especially according to the amount of growth, and the consequent withdrawal of water from below, and its evaporation, chiefly through the plant, but partly also from the surface soil. The amount must obviously also vary very much according to the character of the season.

It may here be observed that supposing the subsoil contained at one time, air equal to one-third of its volume, this would not suffice for the nitrification of as much nitrogen as was taken up for several years in succession by the *Medicago sativa*, or during two years in the case of red clover on the bean-exhausted land. But the nitrogen is not taken up all at once, though most of it will be within a few months of the year, during which period there would be the most active withdrawal of water from below, and evaporation by the plant and surface soil. The replacement of this subsoil water by an equal volume of air would, however, still not suffice. The question obviously arises, how far, or how rapidly, the used up oxygen will be replaced, and on this point there is very little experimental evidence to aid us. We shall refer to the subject again further on.

6. *Can Roots, by virtue of their Acid Sap, attack, and render available, the otherwise insoluble Nitrogen of the Subsoil?*

Thus, then, although the evidence is clear that the nitrogen of raw clay subsoils, which constitutes an enormous store of already combined nitrogen, is susceptible of nitrification, provided the organisms are present, and the supply of oxygen is

sufficient, yet the data at command do not indicate that these conditions could be adequately available in such cases as those of the very large accumulations of nitrogen by the red clover on the bean-exhausted land, and of the increasing and very large accumulations by the *Medicago sativa* for a number of years in succession.

Accordingly, on September 3, 1885, when the holes were open for the soil sampling on the *Medicago sativa* plot, a specimen of the deep, strong, fleshy root of the plant was taken, and on examination it was found that the root-sap was very strongly acid. The roots of three plants were then collected. Of these, No. 1 had four branches, which were respectively—6 feet 4½ inches, 5 feet 10½ inches, 3 feet 6½ inches, and 2 feet 9½ inches in length; No. 2 had two branches—4 feet 10 inches and 2 feet 2 inches in length; and No. 3 had two branches, respectively 3 feet 9 inches and 1 foot 9 inches in length.

The roots were rapidly washed in distilled water, dabbed with clean cloths, weighed, rapidly cut into small pieces, and bruised into a pulp in Wedgwood mortars, with a measured quantity of ammonia-free distilled water. The pulp was then put upon a vacuum filter, and the resulting extract was made up to a given volume with pure distilled water. It was, however, found impossible to get the extract perfectly clear within the limited time it could be exposed to treatment without risk of change, and hence, in these initiative experiments, it was dealt with whilst still somewhat turbid.

The dry matter, ash, and nitrogen were determined in the original root, in the root extract, and in the exhausted root.

The important question was whether the acid root juice would take up nitrogen from a raw clay subsoil such as that from which the *Melilotus leucantha*, the *Medicago sativa*, and the red clover, were supposed to have derived such large quantities in some way. Accordingly, 20 grams of subsoil from the unmanured wheat-fallow plot immediately adjoining the *Melilotus* and *Medicago sativa* plots, were added to a known quantity of the acid root extract in a stoppered bottle, well shaken, and the soil and liquid were left in contact for some weeks, the autumn holidays intervening. It was found, however, on examination, that the extract had lost, and the soil had gained nitrogen, nitrogenous organic matter having been deposited.

In November, 1885, one of the *Medicago sativa* holes was reopened, and fresh quantities of root were collected. These were prepared in substantially the same way as before, but with much greater expedition, more persons being employed. The roots were rapidly passed successively through 4 basins of distilled water, which was renewed as needed.* They were then dabbed with clean dry cloths as before, weighed, cut first into short lengths, and then into very small pieces, the whole being kept in basins covered with glass plates as much as possible to lessen evaporation. The weight was then again taken, a known quantity of pure distilled water added, the whole bruised in Wedgwood mortars, and re-weighed.

* In subsequent experiments it has been found that the wash-water did not become acid during the process.

One weighed portion of the pulp was put on the vacuum filter as before, and another was submitted to dialysis, the diluted pulp being put into a parchment paper sausage dialyser which was then placed in pure distilled water. However, neither the vacuum filtrate nor the dialysate was quite clear. Both these extracts showed a less degree of acidity, and it was evident that the root was now in a very inactive state compared with that of the specimens collected early in September. Various qualitative examinations were made, and it was found that the extracts contained a large amount of nitric acid. It was decided, however, that further detailed investigation of the subject must be postponed until the return of the actively growing period.

It was intended to undertake the subject in the spring and summer of 1886, but owing to the pressure of other work nothing more was accomplished than the comparative testing of the acidity of the sap of the roots of a number of plants representing very various natural families. The matter was, however, again taken up in April, 1887; and, benefiting by previous experience, some advance was made, but still the attempts to entirely free the acid extract from nitrogen were not successful.

It is of interest to observe that the degree of acidity of the sap of the roots collected in April, May, and June, that is during the periods of the most active growth of the season, was considerably higher than in that of the roots collected in September, 1885, after the cutting of the first crop.

The investigation is, however, at present little more than commenced, and any further reference to the results must be postponed to some future occasion.

7. *Action of Dilute Organic Acid Solutions on the Nitrogen of Soils and Subsoils.*

In the autumn of 1885, when it was found necessary to postpone further experiment with the acid root-sap, it was decided in the meantime to examine the action on soils and subsoils, of various organic acids, in solutions of a degree of acidity either approximately the same as that of the *Medicago sativa* root juice, or having a known relation to it. The acids experimented upon were the malic, citric, tartaric, oxalic, acetic, and formic.

In the first experiments, pure water, and dilute solutions of malic, citric, tartaric, oxalic, and acetic acids, were used; each of the acid solutions being of approximately the same degree of acidity as the sap of the *Medicago sativa* roots collected in September, 1885, that is after the removal of the first crop, which represented the greater part of the growth of the season. The subsoil employed was a mixture of the sifted soil of the fifth, sixth, seventh, eighth, ninth, and tenth depths of 9 inches each, from the unmanured wheat-fallow land immediately adjoining the leguminous plots, and contained, as dried at 100° C., 0·047 per cent. of nitrogen.

The mixtures were made in wide-mouthed stoppered bottles, in the proportion of 200 grams of the subsoil to 1000 c.c. of the water or acid solution. After being well

shaken, in the case of the water, the malic acid, and the acetic acid extracts, a given quantity was drawn off at the expiration of one hour. The remainders of these, and the mixtures of the other acid solutions, were frequently shaken, finally left to settle, and after contact for between two and three days the extracts were decanted off, and the soils drained on a vacuum filter. The several extracts being filtered, portions were at once evaporated to dryness on a steam bath, and the nitrogen in them was determined by the soda-lime method. The actual quantity of nitrogen involved in each determination was, however, so small, that recourse was afterwards had to KJELDAHL'S method; and comparative results led to the conclusion that those obtained by it were the more trustworthy. Accordingly, only the general indications obtained by the soda-lime method are here given.

In the case of the water, and of the malic and acetic acid solutions, nitrogen was taken up after 1 hour's contact with the raw clay subsoil; the most being taken up by the malic acid. In each case, after contact for between two and three days, the amounts of nitrogen in the extract were less than after only 1 hour's contact. There had thus obviously been re-precipitation of nitrogenous matter at first taken up, and as the extracts showed scarcely any remaining acidity, the explanation seemed to be that the longer the contact, the more was the acid neutralised by the fixed bases of the subsoil. Of the five organic acid solutions left in contact between two and three days, the malic retained the most nitrogen; next came the acetic and tartaric, then the citric, and lastly the oxalic.

In a second series of experiments, besides the same five acids as before, formic acid was included. The acid solutions were however now twice as strong as those used in the first series. In the case of the malic acid, the periods of contact were 1 hour and 48 hours, and in that of each of the others 1 hour and 24 hours. In each case the acidity of the solution was much reduced by contact with the subsoil, and in each the more the longer the contact. Again the malic acid took up the largest amount of nitrogen; and with the malic, and the formic acids, less was found in the extracts after the longer periods of contact. With the oxalic acid, however, in a striking degree, and less in that of the tartaric, the amount of nitrogen taken up was greater after 24 hours' than after 1 hour's contact, probably owing to the precipitation of the lime in these cases.

It was next decided, with the view of getting larger amounts of nitrogen taken up, to make 3 series of experiments as follows:—

1. With double the quantity of subsoil to a given volume of the acid solution.
2. With double the quantity of acid solution to a given quantity of the subsoil.
3. To add a second quantity of the acid solution to the already once extracted subsoil.

Further, it was decided to experiment with malic acid only; and for comparison with the results on the subsoil, to make parallel experiments with the surface soil of the *Medicago sativa* plot. Lastly, duplicate determinations of the nitrogen in the

extracts were made, one by the soda-lime method, and the other by KJELDAHL'S sulphuric acid method.

In the following Table the results obtained in these experiments by KJELDAHL'S method are given.

TABLE XI.—Showing the amount of the Nitrogen of surface soil and subsoil dissolved by malic acid solution of approximately twice the acidity of the sap of the *Medicago sativa* roots collected in September, 1885.

		Nitrogen dissolved per million soil.	
		After 1 hour's contact.	After 24 hours' contact.
400 grams soil, 1000 c.c. acid solution, each extraction.			
		Per million.	Per million.
Wheat-fallow subsoil	First extraction	2·43	1·82
	Second extraction	2·19	2·19
Lucerne surface soil	First extraction	9·72	8·51
	Second extraction	6·08	7·59
200 grams soil, 1000 c.c. acid solution, each extraction.			
Wheat-fallow subsoil	First extraction	3·28	7·29
	Second extraction	4·03	3·61
Lucerne surface soil	First extraction	8·14	10·81
	Second extraction	4·35	7·31

First as to the experiments the results of which are given in the upper division of the table, in which 400 grams of soil were mixed with 1000 c.c. of acid solution, in each extraction, that is to say, after the removal of the first extract by decantation and the filter pump, a second quantity of the acid solution was added. After 1 hour's contact with the subsoil, the liquid remained only slightly acid, and the amount of nitrogen taken up was very small, representing only 2·43 parts per million of subsoil. After 24 hours' contact the liquid was still less acid, and the amount of nitrogen found in the extract was, calculated per million of subsoil, considerably less than after only 1 hour's contact.

After the addition of a second quantity of the acid solution to the already once extracted subsoil, the liquid remained much more acid than in the case of the first extraction, both after 1 hour's and 24 hours' contact. Still, the amount of nitrogen taken up was very small; being only 2·19 per million soil after 1 hour's, and the same after 24 hours' contact. That is to say, with the greater remaining acidity in the second extraction, there was not less nitrogen taken up after 24 than after 1 hour's contact. It may be observed that, under these conditions, much more total matter, remained dissolved in the second than in the first extract.

Turning now to the parallel results obtained with the lucerne surface soil, which, though poor, still contained about twice and a half as much nitrogen as the subsoil, it is seen that much more nitrogen was found in the extracts than in those from the subsoil. At the same time the liquids after contact showed scarcely a trace of acidity, and they were found to contain much more of other dissolved matters. In the first extraction, after 1 hour 9·72, and after 24 hours 8·51 parts of nitrogen were taken up per million of soil; and in the second extraction 6·08 and 7·59 parts per million. That there was less nitrogen taken up by the second quantity of acid than by the first, is doubtless due to the more readily soluble portion having been already removed. Even in the second extraction of this richer, though still poor, surface soil, about three times as much nitrogen was taken up as from the subsoil.

In the experiments so far considered, nearly the whole of the acid was neutralised in the first extraction of the subsoil, and in both extractions of the surface soil. In the experiments, the results of which are recorded in the lower division of the table, only half the quantity of subsoil or surface soil was mixed with 1000 c.c. of the acid solution; and here, in the case of the subsoil the liquids remained distinctly acid in the first extraction, even after 24 hours' contact, and more strongly acid in the second extraction. In the case of the surface soil, however, in the first extraction the acidity was entirely neutralised, and even in the second extraction nearly so.

The figures show that considerably more nitrogen was taken up, even from the subsoil, when twice the quantity of acid solution was used to a given quantity of it, and when, accordingly, the extracts remained more or less strongly acid. In the first extraction the quantities of nitrogen found in solution were, after 1 hour 3·28, and after 24 hours 7·29, per million soil; that is the more the longer the contact when the liquid remained distinctly acid. In the second extraction, with still greater remaining acidity, the amounts were 4·03 after 1 hour, and only 3·61 after 24 hours. That notwithstanding there was much more remaining acidity, there should be less taken up after 24 hours in the second than in the first extraction, again indicates that a certain quantity of the nitrogen exists in a more readily attackable condition than the remainder. It may be added, that much more mineral matter as well as nitrogen was taken up with the larger proportion of acid solution to a given weight of the subsoil.

With the larger quantity of acid solution to a given weight of the surface soil, much more nitrogen was taken up than under parallel conditions with the subsoil. But, in the first extraction there was little more, and in the second even less, than with twice the quantity of the surface soil to a given quantity of the acid solution. In fact, there was, taking the two extractions together, even less nitrogen taken up with the larger than with the smaller proportion of acid solution to a given weight of soil; but with the larger proportion there was much more mineral matter taken up, whereby the acid would be to a greater degree neutralised. There is, both after 1 hour and after 24 hours, much less nitrogen taken up in the second than in the first

extraction ; again showing that a certain proportion of the nitrogen of the soil is more easily attacked than the remainder.

All the foregoing results illustrating the action of dilute organic acid solutions on the organic nitrogen of soils and subsoils were obtained in 1885, and 1886, by Mr. D. A. Louis, and the strengths adopted had reference to the degree of acidity of the sap of the lucerne roots collected in September, 1885, after the main growth of the season was past. But finding the sap so much more strongly acid in April, 1887, that is at the commencement of the active growth of the season, it was decided to experiment with much stronger malic acid solutions.

The following Table gives the results of experiments made by Dr. N. H. J. Miller ; in which the malic acid solution was of approximately 10 times the acidity of the September, 1885, root-sap, and the mixtures were made in the proportion of 200 grams of the wheat-fallow subsoil to 1000 c.c. of the acid liquid. As before, a portion of the extract was removed after 1 hour's contact, and the remainder after 24 hours'. A second quantity of the acid solution was then added to the already once extracted subsoil, and portions were examined as usual after 1 hour's and after 24 hours' contact.

Table XII.—Showing the amount of the Nitrogen of subsoil dissolved by a malic acid solution of a degree of acidity much greater than that of lucerne root-sap.

		Nitrogen dissolved per million soil.	
		After 1 hour's contact.	After 24 hours' contact.
Wheat-fallow subsoil	First extraction . . .	8·16	13·75
	Second extraction . . .	11·71	7·03

Even in the first extraction more than half the acid remained unneutralised, and a larger proportion still in the second extraction. Under these conditions of constant excess of acid, the raw subsoil gives up considerably more nitrogen, though there was, at the same time, much more mineral matter taken up. In the first extraction the amounts of nitrogen taken up per million subsoil were 8·16 parts after 1 hour, and 13·75 parts after 24 hours ; that is more after the longer contact. In the second extraction, however, less remained in solution after 24 hours' than after 1 hour's contact, from which it would appear that nitrogen once taken up had been deposited.

Obviously the conditions of experiments in which an acid solution is agitated with a quantity of soil are not comparable with those of the action of living roots on the soil. The root action would necessarily affect only a very small proportion of the total soil. But the results recorded clearly show that the greater the acidity of the solution, the more nitrogen is taken up, and the question arises, whether the root

action would not effect more resolution on the surfaces actually attacked? Indeed, this must necessarily be the case if such an action is really quantitatively an important source of the nitrogen taken up by deep and strong rooting plants, with strongly acid sap. In illustration of this necessity it may be stated that, even if as much as 20 parts of nitrogen were taken up per million of soil, as was the case in the last-mentioned experiments in the first and second extractions taken together, this would only represent 600 lbs. of nitrogen per acre to the depth examined, namely 108 inches.

Upon the whole, then, the experiments on the action of weak organic acid solutions on raw clay subsoil, or even on a poor surface soil, have not given results from which any very definite conclusions can be drawn, as to the probability that the action of roots on the soil, by virtue of their acid sap, is quantitatively an important source of the nitrogen of plants having an extended development of roots, of which the sap is strongly acid.

That roots do attack certain mineral substances by virtue of their acid sap, was established by Sachs. He sowed seeds in a layer of sand on polished marble, dolomite, and osteolite, and he found that the polished surfaces were, so to speak, corroded, where in contact with the roots. In regard to these results, Sachs says: ('Text-Book of Botany,' 2nd English edition, p. 702) "every root has dissolved at the points of contact a small portion of the mineral by means of the acid water which permeates its outer cell walls." It was to carbonic acid that Sachs attributed the action in these cases; but there seems no reason to suppose that other acids in the root-sap may not exert a similar action. The results which have hitherto been published have however reference only to the taking up of mineral substances from the soil by virtue of such an action; and so far as we are aware the possibility or probability that the nitrogen of the soil or subsoil is so taken up has not been considered.

Provided it were clearly established that the organic nitrogen of the soil, and especially of the subsoil, was rendered soluble by the action of the acid sap of the root, the question would still remain, whether the nitrogenous body is merely dissolved, and taken up by the plant as such, as the evidence at command seems to show is probable in the case of the fungi, or whether the nitrogenous body, after being attacked by the acid, is subjected to further change before entering the plant? To this point we shall recur presently.

Since the experiments at Rothamsted, above referred to, on the character and the action of the root-sap were undertaken, a preliminary notice of experiments on the nitrogenous organic compounds of the soil has been published by Dr. G. Loges ('Versuchs-Stationen,' vol. 32, p. 201). He found that the hydrochloric acid extracts of soils rich in humus left on evaporation a residue containing a large proportion of the nitrogen of the original soil. In his notice he does not state the strength of the acid used; but from the results it is to be concluded that it was somewhat con-

centrated—indeed of a strength not at all comparable with that of root-sap. Then, the soils, the results relating to which he gives, were extremely rich in nitrogen, and in this respect again bear no comparison with the subsoils from which the lucerne, and other plants experimented upon at Rothamsted, are supposed to have taken up much nitrogen.

Thus, whilst in Loges' experiments, one of the soils acted upon contained 0·804, and the other 0·367 per cent. of nitrogen, the surface soil of the lucerne plot at Rothamsted which yielded such large amounts of nitrogen in the crops contained little more than 0·120 per cent., and the subsoil from which a large quantity of the nitrogen must have been derived, only from 0·04 to 0·05 per cent. Again of the 0·804 per cent. in the one soil, 0·322, or 40 per cent. of the whole was taken up by the acid, and of the 0·367 per cent. in the other soil, 0·083 or 22·6 per cent. of the whole was taken up. In the richer soil the relation of carbon to nitrogen was as 13·78 to 1, and in the other as 11·74 to 1, whilst the relation of carbon to nitrogen in the hydrochloric extract was in the case of the richer soil 6·8 to 1, and in that of the other about 11 to 1.

By phospho-tungstic acid Loges obtained precipitates from the acid extracts, which in the case of the richer soil showed only 6·67, and in that of the other 5·74 carbon to 1 of nitrogen. In reference to these results, it may be observed that this is approximately the relation of carbon to nitrogen in the raw clay subsoil at Rothamsted below the depth at which it is materially affected by manuring or cropping.

Thus, we have found the proportion of carbon to nitrogen to be in the surface soil of rich prairie, or permanent grass land, between 13 and 14 to 1; in that of somewhat exhausted arable surface soil, between 10 and 11 to 1; and in raw clay subsoil about 6 to 1.

Loges states that he has experimented on a great variety of soils, and that he has found in all, without exception, that the hydrochloric acid extract gives the phospho-tungstic precipitate; and he hopes soon to be able to report further on the nature of the highly nitrogenous humic compound obtained. It would thus seem, however, to be an amide or peptone body.

It is of much interest that the nature of the nitrogenous body existing in, or dissolved out of, soils and subsoils should be determined; and to this end it seems desirable to act on soils with stronger acids than those hitherto employed in our own experiments. But results so obtained can obviously have only an indirect bearing on the special question we have in view, namely—whether roots do, by virtue of their acid sap, attack the otherwise insoluble organic nitrogen of the soil and subsoil, and either take it up as such, or bring it into a condition in which it is easily susceptible to further change, and so rendered available as a source of nitrogen to the plant?

Still more recently, MM. Berthelot and André ('Compt. Rend.,' vol. 103, 1886, p. 1101,) have published the results of experiments to determine the character of the

insoluble nitrogenous compounds in soils, and of the changes they undergo, when submitted to the action of hydrochloric acid, of various strengths, for shorter or longer periods, and at different temperatures. The soil they employed contained 0·1744 per cent. of nitrogen. It was therefore much richer than our lucerne surface soil, and about four times as rich as our wheat-fallow subsoil. It was shaken in a flask with water, or dilute hydrochloric acid, in the proportion of 500 grams soil to 1000 c.c. liquid. The clear liquid was just neutralised by potash, then made slightly acid, calcined magnesia added, and the ammonia distilled off, collected, and determined. The remaining liquid was then acidified by sulphuric acid, evaporated to dryness, and the nitrogen determined by the soda-lime method, the result indicating the amount of soluble amide. The following is a summary of their results, which we give in parts of nitrogen per million soil, so as to compare with our own :—

	Nitrogen per million soil.					
	As ammonia.			As soluble amide.		
	18 hours, cold.	5 days, cold.	2 hours, at 100° C.	18 hours, cold.	5 days, cold.	2 hours, at 100° C.
1. Pure water	1·7			8·3		
2. 10 c.c. hydrochloric acid to 400 water (= HCl 3·5 gr.)	4·8	8·75	48·8	27·75	30·25	123·6
3. 50 c.c. hydrochloric acid to 400 water (= HCl 17·5 gr.)	14·4	21·4	101·0	60·6	90·5	356 9
4. 100 c.c. hydrochloric acid to 400 water (= HCl 35 gr.)	14·9	30·4	124·1	68·6	96·5	430·3

The authors call attention to the facts, which are clearly brought to view in the above arrangement of their results, that the amounts, both of ammonia, and of soluble amide obtained, increase with the strength of the acid, the time of contact, and the temperature. They point out that these are products of the action of the acid on certain insoluble nitrogenous bodies in the soil, and that the reaction is similar to that which they have observed in the case of urea, asparagin, and oxamid—that is with well defined amides. The insoluble nitrogenous compounds in the soil are in fact, as previously supposed, amide bodies. They also call attention to the fact that when the clear, filtered, acid extract is exactly neutralised by potash, one portion of the amide still remains soluble, whilst another is precipitated, showing that the amides rendered soluble constitute two groups. The fact of such re-precipitation is quite in accordance with the results obtained in our own experiments, in which less nitrogen remained dissolved after 24 than after only 1 hour's contact, when, with the longer contact, the acidity of the extract became neutralised.

A special point of interest in these results, as compared with those of Loges, is in

the gradation of effect under the varying conditions as to strength, time, and temperature, and in the evidence as to the proportion of the total nitrogen taken up, which is found as ammonia. The proportion of ammonia-nitrogen to amide-nitrogen ranges from about 1 to 5 to 1 to 3, dependent on the conditions. According to the figures, it would seem that the proportion of the total nitrogen dissolved which is determined as ammonia is the greater, the stronger the acid and the longer the contact.

As in LOGES' experiments, so in these of MM. BERTHELOT and ANDRÉ, the strength of acid used was in all cases much greater than that in any of the Rothamsted experiments, and very much greater than is likely to occur in any root-sap. Indeed, not only was the soil operated upon by MM. BERTHELOT and ANDRÉ about four times as rich in nitrogen as the Rothamsted subsoils, but in the most extreme case, that with the strongest acid, and a temperature of 100° C., nearly one-third of the total nitrogen of the soil was dissolved. Hence, although their results are of great interest as indicating the character of the nitrogenous bodies existing in soils, and of the changes to which they are subject when acted upon by acids, they, like those of LOGES, have only an indirect bearing on the question whether by the action of the organic acids of the root-sap, the insoluble organic nitrogen of the soil, and especially of the subsoil, is rendered available as a source of nitrogen to the plant. Supposing this to be the case, as already said, the further question still remains—whether the dissolved amide is taken up as such, or whether it is subject to further change within the soil before serving as food for the plant?

The fact that the formation of ammonia seems to be an essential element in the reaction, points to the conclusion that at any rate part of the nitrogen liberated from the insoluble condition is available in other forms than as soluble or dissolved amide; and, as our experiments show that nitric acid, as well as ammonia, is a constituent of the root-sap, the question arises—whether the liberated ammonia is oxidated into nitric acid before being taken up? Then, again, is the soluble amide taken up as such, or subjected to further change, perhaps first yielding ammonia, and this again nitric acid?

On this supposition we are met again with the difficulty as to the sufficient aëration of the subsoil for such a purpose. It has already been pointed out, that such evidence as exists on the subject clearly shows that the amount of oxygen within the soil at any one time is totally inadequate for the nitrification of the amount of nitrogen taken up by some plants within the season; whilst the replacement by air of the water evaporated would still be quite insufficient. With what rapidity, or to what extent, the oxygen of the subsoil air would be replenished from above as it is used up, there is no experimental evidence at command to show. But whether this would take place adequately or not, it must be supposed that it would occur to some extent.

Turning to the alternative aspect, inasmuch as the insoluble organic nitrogen of the soil exists in the condition of amide bodies, and the chief first product of the action of

acids is soluble amide, it is of interest to consider, whether plants can take up such bodies and assimilate their nitrogen? There can be little doubt that fungi can utilise both the organic carbon and the organic nitrogen of the soil, though they seem to develop the more freely when the humic matters have not undergone the final stages of change by which the compound of so low a proportion of carbon to nitrogen as is found in raw subsoils, has been produced.

8. *Evidence as to whether Chlorophyllous Plants can take up Complex Nitrogenous Bodies, and Assimilate their Nitrogen.*

The first direct experiments to determine whether green leaved plants can take up organic nitrogen were made in 1857 by Dr. (now Sir Charles) Cameron. He experimented with barley, in an artificial soil, and found that when urea was the only soil-source of nitrogen, the plants grew luxuriantly, and took up much of the nitrogen so supplied. Ammonia was not detected in the soil. Hence he concluded that the urea was taken up by the plant as such. No reference is made to nitric acid, and in the absence of evidence to the contrary, it is possible that nitrates were formed, and served as the source of nitrogen to the plants.

In 1861, Professor S. W. Johnson, of Yale, made experiments with maize in an artificial soil. A given quantity of nitrogen was supplied, in one case as uric acid, in a second as hippuric acid, and in a third as guanine. Compared with results in a control experiment without nitrogenous supply, the growth was very greatly increased; and there was no doubt that the substances named had supplied nitrogen to the plants. Professor Johnson states that the conditions of the experiments were not such as to demonstrate that the nitrogenous organic bodies entered the plants without previous decomposition, but from the results of Cameron, and of Hampe, he concludes that this was the case.

In 1865, 1866, and 1867, Dr. W. Hampe ('Versuchs-Stationen,' vol. 7, p. 308, vol. 8, p. 225, vol. 9, p. 49, and vol. 10, p. 175) made several series of experiments, all by the water-culture method. Maize was the plant selected, and the sources of nitrogen supply were—urea, ammonium phosphate, uric acid, hippuric acid, and glycocoll.

At first the experiments with urea were not very successful, apparently owing to an unfavourable condition of the solutions as to mineral supply. Afterwards the plants produced were nearly as good as those grown in a garden; ripe seeds being formed, which, when sown, germinated.

Urea was found in the leaves, stems, and roots. Small quantities of ammonia were sometimes found in the solutions, but only when there was some decomposition of the roots or their excretions, and such formation of ammonia was the most prominent after the blooming. To obviate such formation as far as possible, the solutions were in the later experiments frequently renewed. In corresponding solutions without plants,

ammonia was only found twice throughout the summer. Neither nitrates nor nitrites were ever found in the solutions.

Hampe concluded that the urea was taken up by the plants as such, and that it served as a source of nitrogen to them. This result he considered not inconsistent with the view that plants, other than fungi, cannot utilise products of the plant itself, such as the alkaloids, which they can no more assimilate as a source of nitrogen, than they can sugar as a source of carbon. Urea being, on the other hand, a product of the degradation of animal substance, there seemed no reason why it should not serve as a source of nitrogen to plants.

In his experiments with ammonia Hampe used the phosphate; and small and large maize were the plants selected. In their early stages the plants seemed to suffer rather than to benefit by the ammoniacal supply; but eventually they gave good growth, and produced ripe seeds, which on being re-sown germinated.

In the experiments with uric acid the same descriptions of maize were employed. The solutions were frequently renewed, and in those which were removed ammonia was always found, but not uric acid. But even in solutions without a plant the uric acid rapidly decomposed. From the results it was concluded that the uric acid had served as a source of nitrogen to the plants; though probably not directly, but by its products of decomposition.

In the case of hippuric acid, applied as hippurate of potash, and to the same descriptions of plant, the growth was somewhat dwarf; but seeds, which were found to germinate, were produced. Benzoic acid was always found in the solutions after vegetation, and also in corresponding solutions without a plant. In both cases a mould formed on the surface, but not in the body of the liquid. The question arose whether the benzoic acid was only formed in the solution under the influence of the mould acting as a ferment, or whether in part in the plant itself, glycocoll being at the same time produced, and serving as the nitrogenous supply? If the latter were the case, the action would be the convrese of that which takes place in the animal, when benzoic acid unites with glycocoll, forming hippuric acid, which is eliminated.

Direct experiments were also made with glycocoll itself. With it, the plants were better than in any of the other experiments. At each renewal of the solution, the old liquid was examined both for glycocoll and for ammonia. Glycocoll was always found, but ammonia only in very small quantity, and its occurrence was apparently connected with decomposition of plant-substance. Hampe concluded that glycocoll was as available as nitrogenous food to plants as nitric acid.

In 1868 Dr. P. Wagner ('Versuchs-Stationen,' vol. 11, p. 287) made experiments in continuation of those by Hampe above described. He repeated, with some modifications, the experiments with ammonium salts, hippuric acid, and glycin, and also experimented with kreatin.

With ammonium phosphate good growth was obtained. Neither nitrate nor nitrite

could be found in the plant, and it was concluded that, as in HAMPE'S experiments, the ammonia had served as a supply of nitrogen.

When ammonium carbonate was used, nitric acid was found both in the solution and in the plant; and it was concluded that the ammonia had not served directly as a supply of nitrogen.

In HAMPE'S experiments with hippuric acid, it was proved that it served as a supply of nitrogen to the plant; but as benzoic acid was found not only in the vegetation solution, but in a corresponding solution without a plant, and there was, in both cases, fungoid growth on the surface, it was uncertain whether the breaking up of the hippuric acid had taken place only externally to the plant, under the influence of the fungus acting as a ferment, or also within the plant itself, benzoic acid being excreted. By excluding the access of the air, and by frequently passing carbonic acid through the solutions, the formation of the fungus was prevented. Benzoic acid was, however, still found in the plant-solution, but not in the solution without a plant.

WAGNER concluded that hippuric acid was broken up within the plant itself, benzoic acid being excreted, and that it also suffered decomposition in the solution by the agency of the fungus.

HAMPE had obtained very good growth with glycin, but WAGNER thought it desirable to prevent the formation of the mould on the surface of the solution. This he succeeded in doing by frequently passing carbonic acid through it, and glycin was then easily detected in it. Ammonia was only found when there was some decay of the roots. WAGNER concluded that the glycin had been taken up by the plant as such, and had contributed nitrogen to it.

Kreatin was used as being closely allied to urea, which had been proved to serve as a supply of nitrogen to plants. For some time neither mould, nor ammonia, nor smell, was developed in the vegetation-solution; when they were, it was renewed; and some ammonia again appeared when the roots showed signs of decay. WAGNER could not detect kreatin in the plant, as HAMPE had urea. But from its constant presence in the solution, and the very little development of ammonia, he concluded that it served as nitrogenous food to the plant as did urea.

WAGNER considered it established that the higher plants can obtain nitrogen from complex organic bodies as well as from ammonia and nitrous and nitric acids, and that thus the doctrine of the nutrition of plants was much extended. He did not suppose that such a source was essential, and whether in the case of plants growing in soil such substances would serve as a direct supply would depend on the length of time they could remain in such a medium in an undecomposed condition.

The last experiments of this description to notice are those of W. WOLFF with tyrosin ('Versuchs-Stationen,' vol. 10, p. 13). He had formerly experimented with KNOP, on leucine, tyrosin, and glycocoll; and he now repeated the experiment with tyrosin, to determine whether it served directly, or only by its products of decompo-

sition, as the source of nitrogen. To this end the water-culture method was adopted, and rye was the plant selected.

According to the report, the vegetation went on for more than a year—430 days! The amount of the dry substance produced was 365 times that of the seed sown; but no seed was developed. Neither ammonia nor nitric acid was found in the solutions. But, on boiling, a small quantity of an organic body was deposited. From 4·5 grams tyrosin the vegetation acquired 0·18 nitrogen, corresponding to 2·3 tyrosin. No tyrosin was found in the extract of the stems and leaves, but traces were detected in that of the roots.

W. Wolff concluded that tyrosin suffered change as soon as it entered the plant, and that thus the action differed from that found by Hampe in the case of urea. He considered that the tyrosin was, at any rate in part, transformed in the solution, under the influence of the roots; but that ammonia was not one of the products of the change. If the tyrosin were taken up at all as such by the roots, it did not pass unchanged to the upper organs; but when its nitrogen, in whatever form, was assimilated by the plant, it was distributed through the various organs, as in the case of land plants growing under natural conditions.

From the various results above quoted it seems at any rate very probable, if not absolutely demonstrated, that green-leaved plants can take up soluble complex organic bodies, and assimilate their nitrogen, when they are presented to them under such conditions as in water-culture experiments. Even under such conditions, however, if the nitrogenous substance supplied was readily subject to change in the solution itself, it was doubtful whether it was taken up as such, or only after first undergoing change; and it is pretty certain that such substances supplied to the soil, would either in great part or entirely suffer change before being taken up by the plant.

The probability that the higher plants can, under any circumstances, take up complex nitrogenous bodies, and appropriate their nitrogen, is of considerable interest from a theoretical point of view. But under the ordinary condition of the growth of plants in soil, such substances will seldom if ever be available to them, excepting it may be under the influence of the action of the root-sap in rendering soluble the nitrogenous compounds of the soil and subsoil, which exist in them in an insoluble condition.

It will be of interest next to consider what evidence exists as to other modes in which green-leaved plants may acquire nutriment from compounds existing in an insoluble condition in the soil and subsoil.

Dr. Frank has observed that the feeding roots of certain trees are covered with a fungus, the threads of which force themselves between the epidermal cells into the root itself, investing the cell, but not penetrating the fibro-vascular tissue. In such cases the root itself has no hairs; but there were similar bodies external to the fungus-mantle, which were prolonged into threads among the particles of soil. The

fungus-mantle dies off on the older portions of the root, and its extension is confined to the younger roots—those which are active in the acquirement of nutriment.

This fungus development was always observed in the case of teaks, beeches, hornbeams, and hazels:—in seedlings of 1, 2, or 3 years old, and in trees more than a century old. It was, however, not found on the roots of the associated woodland plants, even when these were growing close to a tuft of the mycorhiza. Nor was it found on the roots of maples, elms, alders, birches, mulberry, buckthorn, planes, walnut, apple, service-tree, hawthorn, cherry, cornel ash, syringa, or elder, &c. Thus, the majority of woodland trees appear to be free, and the occurrence seemed to be almost limited to the Cupuliferæ; though outside of this family the development has nevertheless been observed, as on willows, and some conifers; and it is supposed probable that it may be found to be more general as investigation extends.

In the case of the Cupuliferæ the occurrence seems to be universal. It has been observed in the most widely distant localities, at very different altitudes, in very different aspects, in soils of the most varied geological character, and with very varied amounts of humus, with great variation in the associated herbage, and even in a flower-pot. The growth is perhaps the most luxuriant on chalk soils. It is also the more developed in the first 2 inches, or the richer-in-humus layer of the soil.

The occurrence of a fungus on the roots of certain trees has indeed been recorded before. It has sometimes been considered to be connected with a diseased condition, though it has also been noticed on healthy trees. The observations have, however, not before been generalised.

Frank considers that the conditions are those of true symbiosis. He in fact concluded that the chlorophyllous tree acquires the carbon, and the fungus the water and the mineral matters, that is the soil nutriment.

Frank did not refer to nitrogen. But there is no reason to suppose that the fungus could not, as do the fungi in the case of fairy rings for example, avail itself of the organic nitrogen of the soil.

Here then we have a mode of accumulation of soil nutriment by some green-leaved plants, which so far allies them very closely to fungi themselves. Indeed, it is by an action on the soil which characterises non-chlorophyllous plants, and by virtue of which they are enabled to take up nutriment not available to most green-leaved plants, that the chlorophyllous plant itself acquires its soil-supplies of nutriment. Under such circumstances, it can indeed readily be supposed that the tree may acquire not only water and mineral matter, but organic nitrogen from the soil, and if so probably organic carbon also. In reference to this point, it has already been stated that, from the evidence so far at command, it was concluded that the action is the most marked in the surface layers of the soil rich in humus.

So far as this is the case, it is obvious that such an action of fungi on the soil does not aid us in the explanation of the acquirement of nitrogen from raw clay subsoil by the deep and strong rooted Leguminosæ. Further, it is distinctly stated that the

fungus development in question has not been observed on the roots of any herbaceous plants. It is nevertheless a point of interest, should it be established, that by special means, in special cases, the organic nitrogen of the soil may serve as a supply of nitrogen to chlorophyllous plants. To this point further reference will be made in the course of the discussions upon which we have now to enter.

PART II.

Recent Results and Conclusions of others, relating to the Fixation of Free Nitrogen.

In our introductory remarks it was stated that the object of the present paper was not only to discuss our own results bearing on various aspects of the question of the sources of the nitrogen of vegetation, but to consider the recent results and conclusions of others, and to endeavour to determine how far the evidence yet available is conclusive on the subject. And, as there can be no doubt that the Memoirs of M. Berthelot have materially influenced the course of inquiry in recent years, it will be well to commence with a statement and discussion of his results and conclusions.

1. *The Experiments of* M. Berthelot.

It was, we believe in 1876, that M. Berthelot first called in question the legitimacy of the conclusion that plants do not assimilate the free nitrogen of the air, when drawn from the results of experiments in which the plants were so enclosed as to exclude the possibility of electrical action. More recently he has objected to experiments so conducted with sterilised materials, on the ground that, under such conditions, the presence, development, and action of micro-organisms are excluded. Such objections, if valid, of course put out of court the results and conclusions of Boussingault, ourselves, and others, from experiments so conducted. They at the same time, obviously suggest, though it is true they do not actually necessitate, the adoption of less exact methods of experimenting—methods in which the soils, or plants, or both, are almost unavoidably exposed to accidental sources of unknown amounts of combined nitrogen, and in which the personal equation becomes a very prominent element. At any rate, since the announcement and acceptance of M. Berthelot's objections, numerous experiments have been made without the enclosure of the plants; and results have been obtained showing very various and, in some cases, very large gains of nitrogen, assumed to be due to the fixation of the free nitrogen of the air in some way.

In 1876 ('Compt. Rend.,' vol. 72, pp. 1283–5) M. Berthelot published the results of experiments in which he found that free nitrogen was fixed by various organic compounds, under the influence of the silent electric discharge, at the ordinary

temperature. Such fixation was determined in the cases of benzene, oil of turpentine, marsh gas, and acetylene. In each case a solid nitrogenous body was obtained, from which ammonia was evolved on strongly heating. The electricity was developed by a large Ruhmkorff coil, so that the conditions were comparable with those between the clouds and the ground during a thunder-storm, and the application of the results to vegetation was legitimate for such conditions. He suggests that similar reactions probably take place in the air during storms, and when the air is charged with electricity, organic matters absorbing nitrogen and oxygen.

Again in 1876 ('Compt. Rend.,' vol. 82, pp. 1357–1360), he recurs to the subject. He says that under the influence of the silent electric discharge, nitrogen, whether pure or mixed with oxygen, is fixed by moist filter paper, and by dextrine, to a degree that is very noticeable within a few hours. Neither ammonia, nor any nitrogen acid is a product of the reaction; and thus the fixation may take place in nature without the preliminary formation of ozone, ammonia, or nitrogen acids.

Subsequently ('Compt. Rend.,' vol. 83, 1876, pp. 677–682), he used currents of much weaker tension, more comparable with those incessantly occurring in the air, and the substances experimented upon were moistened filter paper, and a strong solution of dextrine. The tension would correspond to that between the ground and a layer of air two metres above it. The experiments lasted about two months, during which, however, the tension varied considerably, but averaged $3\frac{1}{2}$ elements Daniell. In all cases nitrogen was fixed by the organic substance, forming a nitrogenous compound from which ammonia was evolved by soda-lime.

In 1877 ('Compt. Rend.,' vol. 85, p. 173) he gives further results of the same kind. In experiments in which the difference of electrical potential was not greater than that frequently existing between strata of the atmosphere not far from the ground, he found that filter paper moistened with water and containing 0·010 per cent. of nitrogen, after a month contained 0·045 per cent., whilst similar paper moistened with a solution of dextrine had its percentage of nitrogen raised from 0·012 to 0·192. He considered that his experiments indicated the true explanation of the fixation of nitrogen in nature. The gains are in amount such as would explain how crops acquire the amounts of nitrogen which he considers they must derive from the atmosphere.

In the autumn of 1885 ('Compt. Rend.,' vol. 101, pp. 775–784) M. Berthelot gave the results of experiments on the fixation of atmospheric nitrogen by certain argillaceous earths. He refers to his experiments which established the fact that nitrogen was fixed in some of the immediate principles of plants by the agency of electricity of such feeble tension as is operative all over the globe. He has now to call attention to another mode in which free nitrogen is brought into combination—namely by argillaceous soils under the influence of micro-organisms.

He experimented with two argillaceous sands, and two pure clays—crude kaolins. Some of the experiments were commenced in 1884, but others not until April, 1885;

and for all of these comparative results are given for the period from April 30 to October 10, 1885. It may be mentioned that at the commencement of this period the initial amounts of nitrogen in these materials were very much lower than in any cultivated soils, being respectively 0·0091, 0·01119, 0·0021, and 0·01065 per cent.

Each of these descriptions of soil was exposed under the following conditions :—

1. From 50 to 60 kilog., in open glazed pots, in a closed chamber free from emanations.
2. From 0·08 to 0·10 mm. depth of soil, in open pots, on a trestle 0·7 metre above the ground, in a meadow, with a roof protecting from vertical, but not from oblique rain, or from free air.
3. In similar pots, uncovered, placed on a plank on a tower 29 metres high.
4. 1 kilog. soil, placed in a 4-litre flask, moistened, and closed with a ground stopper; one set being exposed to diffused day light, and a duplicate set kept in a closed cupboard.
5. 1 kilog. of soil put into a 4-litre balloon, heated at 100° C. for 2 hours, steam passed through for 5 minutes, and cooled in filtered air previously heated to 130° C.; then closed and exposed from July 10 to October 6, 1885.

The following tabular statement, summarises the results obtained in the first, second, third, and fourth series of experiments. The upper division shows the actual percentages of nitrogen found, before and after exposure, and the lower division, the gains in the percentage of nitrogen. We give the results in percentages, instead of in parts per kilogram, to compare the better with the figures given relating to our own experiments.

	Initial.	In closed chamber.	In meadow.	On tower.	In closed flasks in light.
Nitrogen found—per cent.					
Yellow argillaceous sand I.	0·00910	0·01179	0·00983	..	0·01289
,, ,, ,, II.	0·01119	0·01639	0·01295	0·01396	0·01503
White clay	0·00210	0·00407	0·00353	0·00557	0·00494
Crude kaolin	0·01065	..	0·01144	0·01497	0·01236
Gain of nitrogen—per cent.					
Yellow argillaceous sand I.		0·00269	0·00073	..	0·00379
,, ,, ,, II.		0·00520	0·00176	0·00277	0·00384
White clay		0·00197	0·00143	0·00347	0·00284
Crude kaolin		..	0·00079	0·00432	0·00171

Thus, although the actual amounts of gain are small, there is in every case some gain. Determinations of nitric acid and ammonia showed that the gains were not

correlative with the amounts of either. Further, calculations showed that the amounts far exceeded those which could be due to ammonia in the air, or to ammonia and nitric acid in the rain; whilst the gains in the closed flasks showed that they could not be due to combined nitrogen from the air or rain.

The author considers the results establish the fact that there is gain of nitrogen quite independently of any absorption of combined nitrogen.

From the evidence so far it might be concluded that the gains in the meadow and on the tower were due to electrical action; but the fifth series of experiments, in which the soils were sterilised by heat, and then left in the balloons from July 10 to October 6, 1885, indicate another influence. In the case of each of the four soils so sterilised, and afterwards exposed, there was, instead of any gain, a slight loss of nitrogen, which was attributed to the heating at the commencement. The cause of the fixation of nitrogen had at the same time been destroyed; nor did the soils recover the power of fixing nitrogen, either by exposure to the air of the chamber, or when a small quantity of the unsterilised soil was added.

It was concluded that there was a fixation of free nitrogen due to living organisms. It was shown that the action was not manifested during the winter, that it was the most effective during the periods of active vegetation, and that it was exercised in closed vessels as well as in the free air.

M. Berthelot estimated that the gains corresponded to gains of nitrogen per hectare of 20 kilog. by sand No. 1, of 16 and 25 kilog. by sand No. 2, and of 32 kilog. by the kaolin No. 3. These estimates are, however, said to be much too low, as they are on the assumption of only 0·08 or 0·10 m. depth of soil, whilst the action extends much deeper. He compares these amounts with 17 kilog. the amount of combined nitrogen in the rain, &c., at Montsouris in 1883; and with 8 kilog., the amount formerly estimated as annually so coming down at Rothamsted; which, however, more recently we have estimated at less than this. On the other hand, taking the amount of nitrogen removed in a crop of hay at from 50 to 60 kilog. per hectare (= 45 to 54 lbs. per acre), he estimates that the loss to the soil will be from 40 to 50 kilog. per hectare (=36 to 45 lbs. per acre). Hence, if it were not for compensation by fixation of free nitrogen, the soil would gradually become exhausted. He considers that the results bring to view not only one of the methods by which fertility is maintained, but that they also show how argillaceous soils, which are almost sterile when first brought into contact with the air, come to yield more and more flourishing crops, and in time become vegetable moulds.

Quite recently, March, 1887 ('Compt. Rend.,' vol. 104, pp. 625 *et seq.*), M. Berthelot has published the results of experiments on the fixation of free nitrogen by vegetable mould supporting vegetation. The experiments were commenced in May, and concluded in November, 1886. He determined the nitrogen by the soda-lime method, and also as nitric acid, in the soil before and after the growth; also in the initial plants (*Amaranthus pyramidalis*), and in the final products. He also determined

the amount of atmospheric ammonia absorbed by sulphuric acid, and the amount of combined nitrogen in the rain; and finally the amount of combined nitrogen in the drainage waters.

The following is a summary of the amounts of nitrogen involved (in grams):—

Initial—In soil 54·09, in rain 0·053, in ammonia of air 0·048, in plants 0·35, total	= 54·541
Final—In soil 56·54, in drainage 0·403, in plants 2·235, total .	= 59·178
Gain	= 4·637

M. Berthelot points out that the gain of nitrogen is nearly equally divided between the soil and the plant, the latter having taken it up from the soil, which he considers is the true source of the gain. He compares the results with those formerly obtained without vegetation thus—

Gains with vegetation	4·64 and 7·58 grams.
Gains without vegetation	12·70 and 23·15 grams.

He assumes that there is with the higher plants, as with animals, a constant loss of nitrogen. He admits however that more evidence is needed absolutely to demonstrate that the plants themselves do not fix, and that they do set free, nitrogen. But he considers it proved by his experiments that vegetable soil does fix free nitrogen; and he thinks it probable that this is the chief source of the gain by the higher plants. Thus, it can be understood how concentrated production exhausts faster than the natural actions restore fertility; whilst in natural vegetation, on the other hand, the fixing of nitrogen may exceed the liberation, and accumulation may thus take place.

Reviewing the whole of these results and conclusions of M. Berthelot, it is in the first place to be observed that whilst the results obtained under the influence of the silent discharge in bringing nitrogen into combination with certain vegetable principles, owed their special interest to the inference that thus free nitrogen might be brought into combination within the plant, he now considers it at least doubtful, whether the higher plants do bring free nitrogen into combination at all, and that probably the gain of nitrogen is by the soil, and not by the plant.

Obviously, if there are organic compounds existing within the soil which have the power of bringing free nitrogen into combination under the influence of electricity of feeble tension, such as occurs in the atmosphere, the soil and not the plant may be the source, and yet the agent be the feeble electric current. So far, however, as it is assumed that nitrogen is so brought into combination in the atmosphere itself, the resulting compound or compounds will be found in the air, and in the aqueous depositions from it; and the extent, or rather the limit, of the amount of combined nitrogen so available over a given area, in Europe at any rate, is pretty well known.

As to the results obtained with soils, with and without vegetation, it must be admitted that M. Berthelot has carefully considered, and endeavoured to estimate, all other apparent sources than free nitrogen. At the same time, the conditions of risk and exposure to accidental sources of gain in the experiments in the chamber, in the meadow, and on the tower, are such that the results could not of themselves be accepted as at all conclusive. To the distinct gains observed in the experiments in closed vessels no such objection can however be raised; whilst the negative results in the sterilised soils constitute another element in favour of the conclusion at which M. Berthelot has arrived.

It is, however, one thing to accept experimental results on the authority of M. Berthelot, and another to adopt his arguments and conclusions in the application of them to the conditions of practical agriculture. To avoid repetition, however, further reference to this part of the subject must be postponed until the results and conclusions of other experimenters have been considered; for, to a great extent, the same facts and arguments are applicable in reference to them, as to M. Berthelot's results and conclusions.

2. *The Experiments of* M. P. P. Dehérain.*

The plan and methods of M. Dehérain's experiments to determine the losses or gains of combined nitrogen were totally different from those adopted by M. Berthelot. They were indeed much on the lines of some of the Rothamsted investigations. He sought to determine the actual losses or gains in the field, under the influence of different manures, of different crops, and of different modes of cultivation. His experiments were made on the farm of the Agricultural School, at Grignon, near Paris, and extended from 1875 to 1885 inclusive. The land had been in lucerne for 5 years, 1870–1874. Four plots were then devoted to each experimental crop as under:—

No. 1 received farm-yard manure, No. 2 nitrate of soda, No. 3 ammonium sulphate, and No. 4 was left unmanured. Each of the manures was applied 3 years in succession, and then the crops were grown for four years more without further manuring.

On one of the sets of four differently manured plots, green maize was grown. On a second set potatoes were grown during the 3 years of manuring, and for two years afterwards, and then wheat for the two remaining years. On the third set beet was grown for 3 years, green maize for 1 year, and then sainfoin for 5 years, and mixed grasses for 2 years, to 1885 inclusive.

The nitrogen was determined in the soil, before the commencement of the experiments in 1875, in 1878 after the three years of manuring and cropping, in 1881 after 4 years cropping without further manuring, and in case of the sainfoin followed by mixed grasses, in 1885 also. Lastly the nitrogen was estimated in the crops. From

* 'Annales Agronomiques,' vol. 8, p. 321, vol. 12, p. 17, and vol. 12, p. 97.

these data, the losses or gains of nitrogen by the soil, during the different periods under the influence of the different manures and crops, were calculated.

M. Dehérain further gives the results of numerous determinations of carbon in the soils, and shows that with a loss of nitrogen there is also a loss of carbon; and that where in the case of the growth of sainfoin, the nitrogen in the surface soil increased, there was not a reduction in the carbon.

In his first paper M. Dehérain summarises his conclusions as follows:—

1. The soil of each experimental plot lost nitrogen from 1875 to 1878 and 1879, when it had grown green maize or potatoes; it also lost when beet was grown.

2. The loss much exceeded the amount due to the removal of the crop.

3. The loss was very sensible even when the soil received abundance of manure, and it continued from 1878 or 1879 to 1881, when the soil grew maize, or potatoes followed by wheat.

4. When, from 1879 to 1881, sainfoin was substituted for beet, not only was loss no longer manifested, but the nitrogen of the soil augmented, and at the same time abundant crops of sainfoin were obtained, which contained large quantities of nitrogen.

5. This nitrogen has not come from the deeper layers of the soil, for these showed an equal, or even rather greater richness in 1881 than in 1879.

With regard to the actual amounts of loss or gain of nitrogen found in M. Dehérain's experiments, the losses especially are extremely large, as the following results will show:—

When farm-yard manure was applied, in very heavy dressings for three years in succession, in amount estimated to supply 400 kilog. nitrogen per hectare per annum (= 357 lbs. per acre per annum), there was, when green maize was grown, a loss of nitrogen by the soil, besides that removed in the crops, amounting to 288 kilog. per hectare (= 257 lbs. per acre) per annum, over the 3 years of the application; when potatoes were grown there was a loss of 242 kilog. per hectare (= 216 lbs. per acre) per annum; and when, with the same manuring for 3 years, beet was grown for 3 years and maize for one year, there was an average annual loss over the 4 years of 679 kilog. nitrogen per hectare (= 606 lbs. per acre).

When nitrate of soda, supplying 192 kilog. nitrogen per hectare (= 171 lbs. per acre) per annum, was applied, the annual loss of nitrogen was, when maize fodder was grown, 401 kilog. per hectare (= 359 lbs. per acre); when potatoes, 436 kilog. per hectare (= 389 lbs. per acre); and when beet was grown for 3 years and maize for one year, the average annual loss of nitrogen by the soil over the four years, besides that removed in the crop, was 557 kilog. per hectare (= 498 lbs. per acre).

When ammonium sulphate was used, supplying annually 252 kilog. nitrogen per hectare (= 225 lbs. per acre), the annual losses were—after the green maize 359 kilog. per hectare (= 321 lbs. per acre), after the potatoes 555 kilog.

(= 496 lbs.), and after the beet and maize 615 kilog. per hectare (= 549 lbs. per acre) per annum.

Lastly, without any manure, the losses were, after 3 years of green maize 379 kilog. per hectare (= 338 lbs. per acre), after 3 years of potatoes 307 kilog. (= 274 lbs.), and after 3 years of beet and 1 year of maize, 476 kilog. per hectare (= 425 lbs. per acre) per annum.

Over the next 4 years, without further manure on the previously manured plots, and still without manure on the previously unmanured plots, the losses of nitrogen by the soil though still large, were generally much less. Besides that in the crops, they were per annum as follows :—

After farm-yard manure, with green maize 133 kilog. per hectare (= 119 lbs. per acre), and with potatoes 2 years and wheat 2 years 308 kilog. per hectare (= 275 lbs. per acre).

After nitrate of soda, with green maize 4 years 206 kilog. per hectare (= 184 lbs. per acre), and with potatoes and wheat, 38 kilog. per hectare (= 34 lbs. per acre).

After ammonium sulphate, with green maize, 148 kilog. per hectare (= 132 lbs. per acre), and after potatoes and wheat 140 kilog. per hectare (= 125 lbs. per acre).

Without manure for 7 years, the annual loss over the last 4 years was with maize 104 kilog. per hectare (= 93 lbs. per acre), whilst with potatoes and wheat there was a gain of 9 kilog. per hectare (= 8 lbs. per acre).

All the losses during the 3 years of the application of the manures, and especially those after 3 years of beet and one year of maize, are far in excess of anything that has come within our own knowledge and experience, and they are in amount such as reflection must show cannot possibly occur in actual practice.

For example, although it is estimated that the farm-yard manure supplied 1200 kilog. nitrogen per hectare (= 1071 lbs. per acre), in the 3 years, and that only 451 kilog. per hectare (= 403 lbs. per acre) were removed in the 3 crops of green maize, leaving a balance of the nitrogen of the manure of 749 kilog. per hectare (= 668 lbs. per acre), yet the surface soil was estimated to lose, not only this amount, but 116 kilog. per hectare (= 104 lbs. per acre) more, or in all 865 kilog. per hectare (= 772 lbs. per acre) in the 3 years. Again, with the same supply, 1200 kilog. nitrogen per hectare (= 1071 lbs. per acre) by manure, in 3 years, and the removal of 561 kilog. per hectare (= 501 lbs. per acre) in beet and maize in 4 years, leaving a balance from the manure of 639 kilog. (= 570 lbs.), the soil is estimated to have lost 2076 kilog. (= 1854 lbs.) more; or in all 2715 kilog. (= 2424 lbs.). It is true that when excessive amounts of farm-yard manure are applied there will probably be some loss by the evolution of free nitrogen, but here the estimated losses amounted to much more than the total nitrogen of the manure after deducting that removed in the crops. Indeed, M. Dehérain calls attention to the fact that, according to the figures, one-fourth of the total nitrogen of the surface soil has been lost! We repeat that such losses certainly do not occur in practical agriculture. But, if such loss could

take place with heavy manuring for 3 years, and the removal of 3 crops of beet and one of maize, what would be the result with ordinary manuring and cropping?

How are these results to be explained? The accuracy of the analytical results recorded by M. DEHÉRAIN may be taken for granted. It seems to us, however, that in the method of taking the samples of soil for analysis, an explanation may be found; and we have the less hesitation in suggesting this, since we have found our own early results obtained under somewhat similar conditions, quite inapplicable for anything like accurate estimates of nitrogen per acre.

Perhaps it is no undue assumption to suppose that there has been more experience of soil sampling at Rothamsted than anywhere else; and we have, accordingly, learnt that very special precautions must be taken, when comparative estimates are to be made of the amount of nitrogen in the soil to a given depth, at different periods. When this is the object, it is absolutely essential that the samples taken should represent very exactly, both the same depth, and the same measure horizontally throughout the depth at the two periods. That is to say, it is essential that they should contain exactly the same proportions of the corresponding layers at the different dates; and this is the more important when the layer to be estimated includes both the manured and worked surface soil, and some of the unmanured and unworked subsoil.

Our own plan is to drive down a square iron frame, without top or bottom, having an exact measure superficially, and the exact depth for which the result is to be calculated. Even when this method is adopted, serious error may arise if at the different periods the soil is in a different state of consolidation, the result of manuring, the working of the land, the cropping, or the seasons. In other words, it is essential that a sample of a given area and depth should contain the same weight of dry soil at the two periods. We have given an illustration of the error possible, and of the correction necessary, when this is not the case, in a paper we published in 1882.*

Now, according to the description of his method given by M. DEHÉRAIN, he adopted the same plan as we did ourselves in our early experiments; that is, he took his samples, not by means of a frame of exact dimensions, but merely with a spade, with which it would be quite impossible to take a sample of exactly the same area throughout the depth adopted. Nor was the depth exactly the same in all cases. It is stated that it ranged from 25 to 30 cm. (= 9·8 — 11·8 inches), whilst the calculations per hectare are made for a depth of 35 cm. (= 13·8 inches). It is obvious, that if the samples were only taken to a depth of 25 or 30 cm., and upon the results obtained the calculations were made for a depth of 35 cm., the amount of nitrogen reckoned per acre, or per hectare, must be too high, as the subsoil from 25 or 30 cm. to 35 cm. deep would doubtless contain a much lower percentage of nitrogen than the layer above the depth of 25 or 30 cm. Indeed, M. DEHÉRAIN's determinations of nitrogen in the subsoils showed less than half as high a percentage as in the surface soils.

* "Determinations of Nitrogen in the Soils of some of the Experimental Fields, at Rothamsted, and the Bearing of the Results on the Question of the Sources of the Nitrogen of our Crops," pp. 32 *et seq.*

We trust, therefore, that M. Dehérain will accept our comments in all friendliness, when we say that our own dearly bought experience leads us to believe that the above facts are quite sufficient to render approximately accurate quantitative estimates at the different periods impossible. From the results, it seems probable that the samples taken at the commencement of the experiments in 1875 were less comparable with those of 1878 and 1879, than were those of these later dates with those of 1881 and 1885. The losses indicated were, indeed, in most cases, much less over the second period of 4 years; a result which is, however, doubtless partly due to the fact that no manure was applied during that period. Another reason for concluding that the samples were less truly representative at the commencement in 1875, than afterwards, is that the percentage of nitrogen found at that date (0·204), is high for the depth stated, of arable soil in ordinary agricultural condition. Though, if the soil is naturally very rich, or if it had been treated otherwise than in ordinary agricultural practice, such a percentage is by no means impossible. The percentages of 0·15 and upwards, as afterwards found, are however quite as high as is usual in good, but long worked arable soil, which is only manured and cropped in the ordinary way.

Then as to the amounts of nitrogen estimated to be gained by the soil to the depth of 35 cm. (=13·8 inches) by the growth of sainfoin for 5 years, and of mixed grasses for 2 years. They were, both on the plot where farm-yard manure had previously been applied, and on that which had been unmanured over the 7 years from the commencement, more than was taken off in the crops of that period. This is certainly more than our own experience would lead us to expect.

From his determinations of the nitrogen in the subsoils at different periods, M. Dehérain concluded that the gains were not from that source. The percentage in the subsoils of the different plots varied however considerably; and on this point it may be stated that in the subsoils at Rothamsted, the variations which are quite independent of manuring and cropping, are so great on the same plot, that we have found it quite impracticable to make calculations as to loss or gain in the total nitrogen of the subsoils.

Upon the whole we conclude, that certainly the estimated losses of the surface soils, and probably also the estimated gains, are higher than can possibly happen in practice; and that the results are due to the method of taking the samples of soil not being such as to ensure strictly comparable estimates at the different periods. At the same time there can be no doubt that there would be losses beyond those due to the removal of the crops, under the conditions in which losses were found; that is, when the land was under arable culture. Nor can there be any doubt that there would be gains in the surface soil when the land was laid down in sainfoin and mixed grasses; and M. Dehérain points out the practical significance of such facts.

M. Dehérain concludes that the loss of nitrogen by arable soil, that is by soil that is mechanically worked, is due to the slow combustion of the nitrogenous organic matter of the soil; the nitrogen being either evolved as free nitrogen, or oxidated

into nitric acid, and carried down into the subsoil, or into the drains. As to the gain by the surface soil, he considers that part is due to the action of deep-rooted plants, in taking up the nitric acid accumulated in the lower layers, and leaving a nitrogenous residue near the surface; a view in which we fully concur. As to gains not so to be accounted for, he considers it not yet settled whether they are due to the ammonia of the atmosphere, as supposed by M. Schlœsing, or to free nitrogen, as supposed by M. Berthelot.

In conclusion it may be remarked that, if the losses in ordinary agriculture were in amount anything like those which M. Dehérain's figures show, even such large gains as are also indicated, would be far from sufficient to compensate them. It would indeed be necessary to seek for other sources of restoration, if our arable surface soils are not to lose their nitrogen much faster than we believe is the case in actual practice. That they do, however, slowly suffer reduction in their stock of nitrogen, when there is no restoration from without, there can we believe be no doubt. In other words, in actual practice, without such restoration from external sources, the losses are not fully compensated.

3. *The Experiments of* M. H. Joulie.*

In this and subsequent sections, we have to consider evidence in regard to the fixation of free nitrogen, obtained, not in closed vessels, nor in the open field, but in vegetation experiments in which the soils and the plants were exposed to the free air, with known amounts of combined nitrogen supplied, and with more or less adequate precautions taken to exclude other sources than the free nitrogen of the atmosphere. M. Joulie's experiments are, in point of date, the earliest of those we have to notice.

M. Joulie made two series of experiments on this subject. In the first he used a sandy-clay soil containing 0·104 per cent. of nitrogen, and in the second a sand containing only 0·0069 per cent. of nitrogen. In each series he used glass pots, with glass pans; in the first series he included 12 experiments, each in duplicate; and in the second series 10 experiments, also each in duplicate. Pounded glass was put at the bottom of each pot, and in each case 1500 grams of the matrix was used. Pure distilled water was supplied to the pans, which also received the drainage; and above the level of the water there were slits in the pots, for the aëration of the soil and the roots. The pots were placed on a bench under a glass roof, and were protected from birds and rain by means of wire gauze; the place of experiment being a court-yard of the Municipal Hospital, Rue Faubourg St. Denis, Paris. The different experiments represented so many different conditions as to manuring, those of Series 1, being as under:—

No. 1.—Without manure.

* 'Bulletin de la Société des Agriculteurs de France,' No. 1, Janvier 1, 1886, pp. 19-29.

No. 2.—A complete mineral manure, containing potassium sulphate and chloride, calcium sulphate, bicalcic-phosphate, and magnesium sulphate.

No. 3.—The complete mineral manure, and nitrate of soda = 0·3 gram nitrogen.

No. 4.—The complete mineral manure, and calcium carbonate.

No. 5.—The complete mineral manure, and caustic lime.

No. 6.—The mineral manure, excluding the bicalcic-phosphate, with nitrate of soda = 0·3 gram nitrogen.

No. 7.—The mineral manure, excluding the potash, with nitrate of soda = 0·3 gram nitrogen.

No. 8.—Farm-yard manure, containing 0·4 gram of nitrogen.

No. 9.—The same quantity of farm-yard manure, and calcium carbonate.

No. 10.—The complete mineral manure, and dried blood = 0·4 gram nitrogen.

No. 11.—The complete mineral manure, dried blood as in No. 10, and calcium carbonate.

No. 12.—Farm-yard manure as No. 8, and mineral constituents sufficient to bring the mineral supply up to that of the complete mineral manure.

On June 30, 1883, 6 germinated seeds of buckwheat were sown in each pot. There was great variation in the luxuriance of growth. On September 6, all the crops were cut at the level of the soil, leaving the roots in it. On September 15, after stirring the soils, each pot was re-sown with a mixture of rye-grass and hybrid trefoil. The rye-grass grew slowly through the winter, but the trefoil almost disappeared. In March, 1884, the herbage was cut, a little more rye-grass and trefoil was sown, and a second cutting was taken on June 18. On June 20 nitrate of soda = 0·1 gram nitrogen was supplied to Nos. 3, 6, and 7 ; and on August 21 the last cutting was taken.

The soils of the duplicate pots were mixed, as also were the crops, and the nitrogen was determined in the soils and in the crops separately. The following Table summarises the results obtained :—

SERIES 1.—Experiments with Sandy-clay Soil.

Experiments.	Manures.	Nitrogen.			Crops, dry.	Nitrogen.			Nitrogen, gain or loss.
		In soil.	In manure.	Total.		In soil.	In crops.	Total.	
		gr.	gr.	gr.	gr.	gr.	gr.	gr.	gr.
1	Without manure	1·56	..	1·56	11·00	1·685	0·3658	2·0508	+ 0·4908
2	Complete mineral manure	1·56	..	1·56	13·45	1·719	0·3540	2·0730	+ 0·5130
3	Mineral manure and nitrate	1·56	0·300	1·86	19·10	1·895	0·5163	2·4113	+ 0·5513
4	Mineral and calcium carbonate	1·56	..	1·56	14·70	1·829	0·3390	2·1680	+ 0·6080
5	Mineral and caustic lime	1·56	..	1·56	13·80	2·042	0·3834	2·4254	+ 0·8654
6	Mineral without phosphate, and nitrate	1·56	0·300	1·86	14·42	1·599	0·4886	2·0876	+ 0·2276
7	Mineral without potash, and nitrate	1·56	0·300	1·86	8·80	1·825	0·3110	2·1360	+ 0·2760
8	Farm-yard manure	1·56	0·400	1·96	14·35	1·752	0·3754	2·1274	+ 0·1674
9	Do., and calcium carbonate	1·56	0·400	1·96	14·85	1·857	0·3679	2·2249	+ 0·2649
10	Mineral, and dried blood	1·56	0·400	1·96	17·95	1·759	0·5564	2·3154	+ 0·3554
11	Mineral, dried blood, and calcium carbonate	1·56	0·400	1·96	12·80	1·523	0·4234	1·9464	− 0·0136
12	Farm-yard manure and mineral	1·56	0·400	1·96	15·65	1·716	0·3814	2·0974	+ 0·1374

In the results given in the Table no account is taken of the nitrogen in the seed sown, which is estimated at not more than 2 to 3 millig. in the buckwheat, and the same in the rye-grass and trefoil, or in all not more than from 4 to 6 millig., which M. JOULIE thinks was largely compensated by leaves of the buckwheat carried away by the wind. He considers that the results are as exact as possible in experiments of the kind. He concludes that, as in M. BERTHELOT's experiments, the results establish the reality of the fixation of free nitrogen in the presence of clay; and further, that the fixation takes place to a greater extent in the presence of vegetation, when the conditions are favourable for the development of the plants.

In the second series of experiments sand instead of soil was used. The percentage of nitrogen in it was only 0·0069, so that the actual amount of combined nitrogen supplied in the 1500 grams put into each pot, was only 0·1035 gram.

This series included 10 conditions as to manuring, each in duplicate. Each pot of experiments 1 to 8 received the complete mineral manure—No. 1 alone; Nos. 2, 3, 4, and 5, each with 0·3 gram of nitrogen as nitrate of soda; No. 6 with 0·2 gram nitrogen as nitrate of soda (half applied at the commencement and half a month later); No. 7 with 0·2 gram nitrogen as ammonium sulphate; No. 8 with 0·3 gram nitrogen as dried blood; No. 9 received farm-yard manure = 0·3 gram nitrogen, with mineral constituents sufficient to bring the mineral supply up to that by the complete mineral manure; lastly, No. 10 received at the commencement 0·3 gram nitrogen as powdered hay, and later 0·1 gram as nitrate of soda; the mineral composition of the manure being made up as in the case of the farm-yard manure.

On May 25, 1884, 10 germinated seeds of buckwheat were sown in each of the pots, excepting those of Experiment 2, which received only 5, and those of Experiment 3 which received 15. On September 16 the plants were cut, and they and the soils were analysed, the duplicates being mixed as before. The results are given in the following Table.

SERIES II.—Experiments with Sand as Soil.

Experiments.	Manures.	Nitrogen.			Crops, dry.	Nitrogen.			Nitrogen, gain or loss.
		In Soil.	In Manure.	Total.		In Soil.	In Crops.	Total.	
		gr.	gr.	gr.	gr.	gr.	gr.	gr.	gr.
1	Complete mineral manure	0·1035	..	0·1035	0·970	0·1455	0·0290	0·1745	+ 0·0710
2	Do., and nitrate soda	0·1035	0·300	0·4035	6·825	0·3270	0·1405	0·4675	+ 0·0640
3	Do., do.	0·1035	0·300	0·4035	6·585	0·3060	0·1680	0·4740	+ 0·0705
4	Do., do.	0·1035	0·300	0·4035	5·890	0·4080	0·1375	0·5455	+ 0·1420
5	Do., do.	0·1035	0·300	0·4035	7·850	0·2280	0·2525	0·4805	+ 0·0770
6	Do., do.	0·1035	0·200	0·3035	7·612	0·2280	0·2420	0·4700	+ 0·1665
7	Do., and ammonium sulphate . .	0·1035	0·200	0·3035	6·425	0·1850	0·2315	0·4165	+ 0·1130
8	Do., and dried blood	0·1035	0·300	0·4035	5·600	0·2685	0·1375	0·4060	+ 0·0025
9	Do., and farm-yard manure . . .	0·1035	0·300	0·4035	4·572	0·4350	0·1205	0·5555	+ 0·1520
10	Do., powdered hay, and nitrate soda	0·1035	0·400	0·5035	1·225	0·3570	0·0390	0·3960	− 0·1075

In the experiments of the first series, with a range of from 1·56 to 1·96 gram of combined nitrogen supplied, there were, in several cases, indicated gains of 0·5 gram, or more; and in one case the gain amounted to 0·865 gram. In the experiments of the second series, with a total supply in sand and manure generally ranging from 0·3 to 0·5 gram, there was, in one case a loss, in five cases the gain was less than 0·1 gram, and in no instance did it reach 0·2 gram. M. Joulie attributes the less amount of gain in the second series, to the much shorter period of vegetation involved.

Reviewing the results of the two series of experiments, M. Joulie says the variable quantities of nitrogen gained cannot be attributed to dust, ammonia, or other compounds of nitrogen, in the air, as all the pots were equally exposed to these; whilst there is a range from 0·1075 gram loss, to 0·8654 gram gain of nitrogen, the difference amounting to nearly 1 gram. The result must be due, therefore, to the fixation of the free nitrogen of the air, either in the soil or by the plant. M. Berthelot attributed the result in his experiments to the clay soil, under the influence of microbes; but M. Joulie cannot go so far. It was, however, true that, in his experiments, the surface of the water in the pots and the surface of the soils showed myriads of microbes. He asks—if such bodies can cause the fixation of free nitrogen, why should not grouped cells, as in the case of the higher plants, have the same power?

He further says—as plants have the power of causing the combination of carbon with the elements of water, after having decomposed carbonic acid, whilst chemists can only reduce it to carbonic oxide; as MM. Thenard have succeeded in bringing nitrogen into combination with the elements of water; and as M. Berthelot has shown that free nitrogen is brought into combination with dextrine and cellulose under the influence of the silent electric discharge—it is only a logical consequence that free nitrogen should be brought into combination within the plant. In reference to this argument it may be oberved that the parallelism of the action by which free nitrogen combines with the elements of water in the laboratory, with that by which carbon and the elements of water combine within the plant, only holds good on the assumption that the carbon of the carbonic acid is first reduced to the free state, and so combines with the elements of water, without the intervention of its own oxygen.

M. Joulie compares the amounts of nitrogen fixed in his various experiments with the amounts of crop produced, and observes that the gains have no relation to the amount of vegetation. He next comments on the connection between the condition of manuring of the various soils, and the amounts of nitrogen gained. Referring to the results of the first series of experiments, he points out that whilst without manure the gain was 0·491 gram, it was raised to 0·513 gram by purely mineral manure. Again, whilst the addition to the mineral manure of 0·3 gram nitrogen as nitrate of soda only gave a further gain of 0·038 gram, and the addition of calcium carbonate increased the fixation by only 0·0950 gram, the addition of caustic lime increased the fixation by 0·352 gram. It appeared, therefore, that lime exercised a very favourable influence on the phenomenon.

On the other hand, the use of organic matter, as farm-yard manure, or dried blood, much reduced the amount of fixation. The addition of calcium carbonate to the farm-yard manure increased the fixation, whilst the same addition to the dried blood reduced it.

When in the first series of experiments either phosphoric acid or potash was excluded from the mineral manure, there was a most remarkable decline in the amount of fixation, indicating, M. JOULIE thinks, how necessary is a due balance of the mineral supplies for the full development of the action.

In the second series, as in the first, the unfavourable influence of organic manures was obvious.

M. JOULIE concludes that his own results, like those of M. BERTHELOT, show that the fixation of nitrogen is due to a physiological action. Microbes play an important part; and his own experiments show that the action is developed in the absence of clay. His results were not very favourable to the supposition that the plants themselves effected the fixation; but he considers that further comparative experiments, with and without vegetation, are necessary to settle the point. For the present he limits himself to the establishment of the great fact of the fixation of the free nitrogen of the atmosphere, leaving to the future the exact explanation.

In order to show the practical importance of the fixation of free nitrogen, M. JOULIE takes for illustration the results of the experiment No. 5, in the first series, in which the largest amount of gain was indicated. In that experiment the complete mineral manure, with caustic lime in addition, was used, without any artificial supply of nitrogen. At the commencement the soil contained 1·56 gram, and at the conclusion 2·042 grams of nitrogen, and the crops contained 0·3834 gram, showing a total gain, therefore, of 0·8654 gram nitrogen. As the soil in the pots was 10 cm. deep, he calculates that this would correspond to 1144 kilograms of nitrogen fixed per hectare weighing 2000 tonnes (= 1021 lbs. nitrogen per acre). Or, reckoning only according to the relative superficies of the soil in the pots, and of a hectare, the gain of nitrogen would be 432 kilograms per hectare (= 386 lbs. per acre). He further reckons, that the value of the nitrogen gained, at 1 franc 50 per kilogram as in manures would be 1716 francs, or 650 francs per hectare (= 555 or 210 shillings per acre), according to the mode of calculation adopted. He admits, however, that it cannot be estimated so high, because the nitrogen fixed in the soil is in a form not at once assimilable by plants.

In reference to the above results, M. JOULIE says that our own at Rothamsted, and those of M. DEHÉRAIN in France, obtained in field experiments, cannot be relied upon as the basis of conclusions on this subject; because the samples of soil taken at different times cannot exactly represent the mean composition of the soil, and because the layer of soil sampled may have lost combined nitrogen by drainage, or gained it from the subsoil. M. JOULIE, on a former occasion, indicated in more detail his

objection to our mode of experimenting; but he did so in a way which showed entire ignorance or misconception of our method.

We have already given our reasons for believing that certainly the losses, and probably the gains also, shown in M. DEHÉRAIN's experiments were too high. We nevertheless, quite agree that there would be losses where he found losses, and that there would be gains where he found gains. It is to be observed, however, that it was under the conditions of arable culture, that is of artificially aërated soil, and with vegetation, that M. DEHÉRAIN found great losses, whilst it is in well aërated soils, also with vegetation, that M. JOULIE finds such enormous gains.

It is further to be observed that the large gains shown in M. JOULIE's results were obtained chiefly in the growth of buckwheat, and not with plants of the Leguminous family which are reputed to be "*Nitrogen collectors.*" From our own results, taken together with known facts as to agricultural production, and the fertility of soils, it may be confidently affirmed that such gains as M. JOULIE finds within a period of about 14 months, do not take place, either with or without vegetation, in ordinary soils, in ordinary practice.

4. *The Experiments of* Dr. B. E. DIETZELL.

At the meeting of the *Naturforscher-Versammlung* at Magdeburg in 1884, Dr. DIETZELL gave the results of experiments, the primary object of which was to determine whether plants absorb combined nitrogen from the atmosphere by their leaves; but they equally afford evidence on the question whether they assimilate the free nitrogen of the air. The plants selected were peas and clover, each of which he grew under four conditions as to manuring. A garden soil, containing 0·415 per cent. of nitrogen was used, and the experimental pots were made of hard burnt clay. The plants were watered with distilled water, and the drainage was returned to the soils. The pots and their contents were exposed to free air, but protected by a linen roof.

The conditions of the different experiments were as follows:—No. 1, without manure; No. 2, manured with kainite; No. 3, with kainite and superphosphate; No. 4, with kainite, superphosphate, and calcium carbonate; No. 5, with kainite, superphosphate, and calcium carbonate, but without a plant; and No. 6, without either manure or plant.

The nitrogen was determined in the original soil, and in the seed; also in the soil at the conclusion of the experiment, and in the plants grown. The following figures show the losses or gains of nitrogen, represented in percentage of the original nitrogen in soil and seed:—

	Loss or gain of nitrogen.		
	Peas.	Clover.	Without plant.
	per cent.	per cent.	per cent.
Without manure	− 10·69	− 5·10	+ 0·26
Kainite	− 15·32	− 14·76	
Kainite and superphosphate	0·00	− 7·37	
Kainite, superphosphate, and calcium carbonate	− 12·72	− 10·38	− 10·24

Thus, there was, in every case but one with the peas, and in every case with the clover, a loss, not a gain, of nitrogen. There was also a loss where the soil was manured, but left without a plant.

On the other hand, in the experiment without either manure or plant, the figures show a gain of nitrogen. In reference to this last result, it should, however, be stated, that whilst in one German account it is, as in the Table, given as a gain of only 0·26 per cent. of the original nitrogen, in another German account, as well as in an English one, it is represented as a gain of 0·26 gram. In the first case the gain would be immaterial, whilst in the other it would be considerable, though still but small compared with the results obtained by M. Joulie.

It is to be observed that whilst with almost exclusively non-leguminous growth, M. Joulie found gains of nitrogen in all cases, and in some very large gains, Dr. Dietzell, experimenting exclusively with leguminous plants, which are credited with being beyond all others atmospheric nitrogen accumulators, in all cases found losses instead of gains. How is this discrepancy to be explained? It may be answered, that with a garden soil containing so much as 0·415 per cent. of nitrogen, it is not at all surprising that there should be some loss. Indeed loss would seem to be a perfectly natural result; and it is obvious that, neither from the combined nitrogen of the atmosphere, or that due to accidental sources, nor from free nitrogen, either directly or indirectly, did these reputed nitrogen-collectors gain nitrogen enough to compensate the losses from the rich soil. It is, indeed, recorded gains that require confirmation, with very careful methods of experimenting, before they can be accepted as conclusive evidence of the fixation of free nitrogen, and not as due merely to accidental sources of combined nitrogen, or to other experimental errors almost inevitable in experiments in which the soils and the plants are not enclosed, but exposed to the free air.

In conclusion, all the results of Dr. Dietzell, excepting the one in which he found a gain, seem quite accordant with well established facts. On the other hand, if free nitrogen is really fixed in the soil under the influence of microbes, it certainly might be supposed that the result would be developed in a soil so rich in organic matter, and doubtless, therefore, in micro-organisms also, as a garden soil containing 0·415 per cent. of nitrogen; and especially might it be supposed that it would be developed in

the presence of leguminous growth, in connection with which, if at all, the establishment of the reality of such an action would serve to explain facts as yet not otherwise fully explained.

5. *The Experiments of Professor* B. Frank.

In the number of the 'Berichte der Deutschen Botanischen Gesellschaft' for August, 1886, Dr. Frank gave a paper, "Ueber die Quellen der Stickstoffnahrung der Pflanzen." At the meeting of the *Naturforscher-Versammlung*, held at Berlin, in September, 1886, he gave a further communication on the subject; and he has since published a paper on the position of the question, before, at, and after that meeting.

He admits the probability of the conclusion of Boussingault and others, that plants do not directly assimilate free nitrogen. He states, however, that in practical agriculture it is assumed that some plants do fix the free nitrogen of the air; and he refers to the experience and writings of Schultz-Lupitz, and others, on the point, especially during the last 10 years. Thus, Schultz-Lupitz found that certain Leguminosæ, especially lupins, grew well in a poor soil, under the influence of mineral manures; and so far from appearing to exhaust the soil, cereals, roots, and potatoes, grew well after them, as they would if nitrogenous manures had been applied.

Frank refers to the amount of combined nitrogen coming down in rain, &c., as about 3 kilograms per hectare (= 2·7 lbs. per acre), per annum, and to the average amount of nitrogen removed in crops as 51 kilograms per hectare (= 45·5 lbs. per acre), apparently obtained from the air by the nitrogen-gathering plants, which are considered more effective than manure and cattle feeding. He points out that the evidence is not conclusive, and he recognises that the question is, whether this is only "*Raub-bau*" after all? This can only be settled by direct experiments.

He had been working at the subject for three years, and now gives the results of the last completed experiments, those of 1885. The first question to be decided was—do the so-called nitrogen-gathering plants enrich the soil, whilst the same soil, with the same exposure, but without a plant, does not gain combined nitrogen?

He experimented with a humus-sand soil, finely sifted. In some cases he used cylinders of pottery glazed inside, 80 cm. (= 31·5 inches) deep, and 17·5 cm. (= 6·9 inches) wide; in others glass cylinders, also of 80 cm. deep, but only 11 cm. (= 4·3 inches) wide. To the rim of each cylinder a cap of wire gauze was fixed, to exclude insects; and the vessels were exposed to free air. The soils were watered with distilled water. One of the wide earthen cylinders, and two of the narrow glass cylinders, were left without a plant. In one wide earthen cylinder three lupin seeds were sown; in one narrow glass cylinder two lupin seeds, in another one lupin seed only, and in another one lupin seed and 20 incarnate clover seeds were sown. If weeds grew where there was no experimental plant, they were stocked up, but if in the vessels with the experimental plants, they were undisturbed.

The nitrogen was determined in seed, in the products of growth, and in the soils before and after growth. The seeds and plants were dried at 50° C. for analysis, and it was found that the lupin seeds contained an average of 0·009 gram. nitrogen per seed, and that 20 incarnate clover seeds contained 0·003 gram of nitrogen.

Summary of Dr. FRANK's results in the summer of 1885.

	Nitrogen.						
	Per cent. in soils.		Total in soil and seed at commencement.	In the plants.	Total in soil and plants at conclusion.	Gain or loss on the original quantity.	
	Before experiment.	After experiment.				Actual.	Per cent.
	Per cent.	Per cent.	Grams.	Grams.	Grams.	Grms.	Per cent.
1. Earthen cylinder without plant	0·0957	0·0907	20·5755	—	19·5112	−1·0643	− 5·1
2. Glass cylinder without plant .	0·0957	0·0837	9·2589	—	8·0979	−1·1610	−12·5
3. Glass cylinder without plant .	0·0957	0·0832	7·1775	—	6·2400	−0·9375	− 8·69
4. Earthen cylinder with 3 lupin seeds	0·0957	0·1065	20·5547	0·8208	23·6758	+3·1211	+15·2
5. Glass cylinder with 1 lupin seed	0·0957	0·0992	9·0373	0·1138	9·4781	+0·4408	+ 4·87
6. Glass cylinder with 1 lupin, and 20 incarnate clover seeds	0·0957	0·0854	8·29039	0·2295	7·6169	−0·67349	− 8·08
7. Glass cylinder with 2 lupin seeds	0·0957	0·0893	8·8032	0·0274	8·2251	−0·5781	− 6·56

It is seen that the actual amounts of nitrogen involved were large, being about 20 grams in the experiments in the wider vessels, and nearly half as much in those in the narrower vessels. The losses were in some cases about, or more than, a gram; and one of the two gains amounted to more than 3 grams.

In each of the three experiments without a plant (Nos. 1, 2, and 3), there was a loss of nitrogen. FRANK states that it was not as nitric acid, which either diminished but little, or increased, there being no plant to take it up. Nor was it as ammonia, as a direct experiment with a rich peaty soil, enclosed under a luted bell jar, and ammonia free air passed through it, showed very little ammonia evolved. He points out that the more imperfect the ventilation of the soil, the less was the gain; and he considers it probable that in the absence of ventilation the evolution of free nitrogen would be enhanced. In fact the losses were greater in experiments 2 and 3 with vessels 80 cm. (= 31·5 in.) deep, and only 11 cm. (= 4·3 in.) wide, than in No. 1 with a width of 17·5 cm. (= 6·9 in.).

It is indeed obvious that, with vessels so narrow and deep, and closed at the bottom, as according to the description we conclude they were,* and with no plant to cause evaporation, and with consequently very little aëration, the conditions would be favourable for the evolution of free nitrogen, and the more so in the narrower vessels. In fact it may be doubted whether, if there had been holes at the bottom of the

* We have since ascertained that the vessels were closed at the bottom.

vessels, and free aëration had been kept up, there would have been any loss at all from a soil containing, as this soil did, less than 0·1 per cent. of nitrogen.

It is further to be observed that the losses with growth were, in No. 6, where the one lupin plant died before blooming, and where only 7 of the 20 incarnate clover seeds grew, and in No. 7 where only one of the two lupin seeds grew, and it gave very small produce. In both cases, therefore, there would, independently of the soil itself, be decomposing organic matter, conditions under which, in the experiments of Boussingault, and also in those at Rothamsted, there was more or less loss, supposed to be as free nitrogen.

Again, in the experiment above referred to, made to determine whether there was any material loss as ammonia, Frank used a very unusually rich soil, containing 1·1836 per cent. of nitrogen, which after exposure for 180 days to a current of air in shallow vessels, only contained 1·0976 per cent. It had lost therefore 0·0860 per cent., corresponding to 7·27 per cent. of its total nitrogen, of which only 0·0004 was as ammonia. In reference to this loss Frank says that if such loss is always going on in the soil, we must suppose that there is restoration in some way. But it is to be observed that the soil in which he found such loss was not only about 12 times as rich as the one used in his other experiments, but probably 8 or 10 times as rich as the majority of ordinary arable soils. Hence it is obvious that the amount of loss it sustained, cannot be taken as any indication of what happens in actual practice. Nor can the conditions of the experiments in the narrow and deep vessels without ventilation, be considered comparable with those of ordinary arable surface soils, or even of subsoils with fairly good natural, or with artificial drainage.

That soils do lose nitrogen, not only by the removal of crops, but also by drainage of nitric acid, is certain; and if there is no return of nitrogenous manures from without, the result is a gradual diminution of the fertility, so far as the nitrogen is concerned. But the balance of evidence is against the supposition that there is a constant and considerable loss by the evolution of free nitrogen, in the case of arable soils which are only moderately rich in nitrogenous organic matter, and which are fairly drained, either naturally or artificially.

On this point it may be mentioned that, in those of the field experiments at Rothamsted in which the unusual practice of applying farm-yard manure every year is adopted, it is found that there is considerable loss of nitrogen from the soil, beyond that known to be removed in the crops, and estimated to be lost in the drainage. On the other hand, where no nitrogen has been applied for many years, and the amount of nitrogen in the surface soil is only about, or little more than, 0·1 per cent., the loss of nitrogen by the soil over a long series of years corresponded approximately with the amounts removed in the crops, together with those estimated to be lost in the drainage. Again, when ammonium-salts are applied, even so late in the season as October or November, and drainage takes place soon afterwards, the drainage-waters will contain amounts of nitrogen showing a very direct relation to the different

amounts of ammonia applied in the manure; but scarcely any of it as ammonia, nearly the whole existing as nitric acid; and this is the case although the drainage passes through 20 inches or more of raw clay subsoil. Lastly, direct experiments have shown that there is a diminution in the amount of nitric acid in the soil down to a certain depth, varying according to the root-range of the crop grown, and to the season, but that in the depths of the subsoil below this point, the amount is again greater.

Upon the whole, then, we are disposed to think that, in most arable soils which are only manured and cropped as in ordinary practice, and which have fair natural or artificial drainage, there is little if any loss by the evolution of free nitrogen.

We would indeed submit, that the losses found by Dr. FRANK in his series of 7 experiments, are in all probability largely, if not entirely, accounted for by the special conditions of the experiments themselves to which attention has been called, and that those found in the rich soil, in the closed vessel, depended greatly if not wholly on the abnormal character of the soil itself.

The gains (as in Experiments 4 and 5) are, however, by no means so easy to explain. Indeed, if there were no accidental source of error, such as all vegetation experiments in free air must be more or less liable to, the explanation obviously would be, that the free nitrogen of the air had come into play in some way.

Dr. FRANK supposes that, even in Experiments 6 and 7, where a loss was indicated, there had nevertheless been a gain under the influence of the plant growth, but not sufficient to counterbalance the loss. We would suggest that, in Experiments 4 and 5, where a gain was indicated, there may have been no loss at all; especially in No. 4, with the wider vessel, and where the growth of the lupins was the most luxuriant, and the seed ripened; for, under such conditions, there would be much more evaporation, and therefore much more movement within the soil, and aëration of it.

But, apparently giving full force to the evidence in his experiments of loss by the evolution of free nitrogen, and taking it as confirmation of the supposition that in actual practice soils suffer to a very material extent in this way, Dr. FRANK says that all that can be concluded with certainty is—that two opposite actions are at work in the soil—one setting free nitrogen, and the other bringing it into combination—the latter being favoured by the presence of vegetation. He admits that neither his own results, nor those of others, afford decisive evidence as to how this takes place; nor does he think that it follows from his results, that the plant itself effects the combination.

Independently of direct experimental evidence on the point, he considers it unlikely that the gain of nitrogen can be due to the ammonia of the air, because it is so small in amount, because the gain is by the soil rather than by the plant, and lastly, because, as the ammonia of the air is largely due to emanations, if it were the source we should be without explanation of the circulation of nitrogen in nature; that is, of the return of free nitrogen into combination, to compensate for the losses by its evolution from combination.

Indeed, with Dr. FRANK, as with other investigators of this subject, a prevailing idea seems to be, that there must exist a source of compensation for the loss of combined nitrogen by the removal of crops, by drainage, and above all by the evolution of free nitrogen from the soil, and in other ways. We believe, however, that the losses by the removal of crops are much exaggerated, due account not being taken of the return by the manures of the farm; also that the loss by the evolution of free nitrogen by the soil is exaggerated, the results obtained in the laboratory not being comparable with the conditions in the field. At the same time we believe, that such losses as do in reality take place in ordinary agriculture, are not fully compensated; but that arable soils yielding products for sale, and not receiving nitrogenous manures from without, do gradually reduce in fertility. so far as their nitrogen is concerned.

6. *The Experiments of Professor* HELLRIEGEL *and Dr.* WILFARTH.

At the Berlin meeting of the *Naturforscher-Versammlung*, held in September, 1886, in the Section *Landwirthschaftliches Versuchswesen*, Professor H. HELLRIEGEL gave a paper entitled, "Welche Stickstoffquellen stehen der Pflanze zu Gebote?" One of ourselves was presiding at the time, and the communication was obviously considered by the numerous agricultural chemists present to be one of great interest and importance. We have vainly tried to get the paper *in extenso*;* but we have now two accounts of the results, one by HELLRIEGEL himself, in the 'Zeitschrift des Vereins für die Rübenzucker-Industrie des Deutschen Reiches,' and another in a very comprehensive summary of the evidence relating to the sources of the nitrogen of vegetation, published by Professor KÖNIG.†

HELLRIEGEL first gave results of experiments with barley, oats, and peas, made in pure washed sand, in pots 20 cm. deep, each containing 4 kilograms of the material. Nutritive solutions containing no nitrogen were added to all. One series of pots received besides, a fixed quantity of nitrogen as nitrate of soda, a second twice as much, and a third four times as much. The results showed that in the case of the gramineous plants the amount of produce grown had a direct relation to the quantity of nitric nitrogen supplied. It was very different with the peas.

In many comparative experiments he got astonishing growth with peas in the sand with all other food substances, but without nitrogen, whilst under exactly similar conditions the Gramineæ showed nitrogen-hunger and failed. He gives the following results with peas:—

* In an interview with Professor HELLRIEGEL, at the meeting of the *Naturforscher-Versammlung*, held at Cologne in September, 1888, we learnt that the details of his experiments have not yet been published, but that a full paper is in course of preparation. [*October*, 1888.]

† 'Wie kann der Landwirt den Stickstoff-Vorrat in seiner Wirtschaft erhalten und vermehren?' Berlin, 1887.

	Total above-ground dry substance.	Seed produced.
	grams.	grams.
1884	28·483	13·947
1885	27·816	11·710
1885	33·147	12·426
1886	20·372	8·956

To convey an idea of what 33 grams of dry produce means, he states that with barley that amount was never obtained even with the addition of nitrates. So far as we know, he has not estimated the amount of nitrogen in the produce,* which, however, would be large ; and as to the source of it, he says it is obviously from the air.

The quartz sand was washed many times, the nutritive mixture given contained no compound of nitrogen, and the plants were watered with distilled water (the first third of the distillate not being used). Hence he considered the supposition that accidental impurity was the source of the nitrogen to be out of the question, especially when the amount of the produce is considered. Further, the constant failure of the Gramineæ under exactly the same conditions, afforded direct proof that the soil was not the source of the nitrogen. He concludes :—

The Papilionaceæ are distinguished from the Gramineæ in not being dependent on the soil for their nitrogenous food. The sources of nitrogen which the atmosphere affords have, for these plants, the highest importance. They alone can suffice to bring them to a normal or full development.

To determine how far the combined nitrogen in the atmosphere was the source, he made a series of 4 experiments, in each of which a pot of peas was enclosed under a glass shade, and a constant stream of air was passed through ; in No. 1, without first washing the air, but in Nos. 2, 3, and 4, the air was washed to remove all nitric acid and ammonia. The result was, that the growth was as good with the washed as with the unwashed air. There only remained, therefore, the supposition that the Papilionaceæ have the power of utilising free nitrogen. He accepts the conclusion of Boussingault that the Papilionaceæ cannot directly assimilate free nitrogen ; but the possibility of an indirect action is not thus excluded. In the first place Berthelot had shown that bacteria abound in the soil, and possess the power of bringing free nitrogen into organic combination ; and secondly the nodules found on the roots of normally growing Papilionaceæ are full of bacteria.

The author has often observed that when peas are grown in a nitrogen-free soil, the growth is quite normal, and the colour of the leaves quite healthy, until the reserve material of the seed is used up. Growth is then arrested, and the leaves become pale or yellow ; but after a shorter or longer time, they regain their green colour, a second period of growth begins, and it continues to the end. In a series of parallel experiments, however, some plants will develop full, and others only a very limited growth.

* See foot-note, p. 64.

Examination showed that the plants which did not develop beyond the first period, had either no nodules on their roots, or only weak indications of them; whilst the roots of the plants which developed favourably, had the nodules, and the more, or the older and stronger, the nodules, the better was the development of the plants. He, therefore, instituted experiments to determine whether by the supply of the organisms the formation of the root nodules and favourable growth could be induced; and on the other hand, whether by their exclusion the result could be prevented.

To each of ten out of 40 experimental pots, with nitrogen-free soil, 25 c.c. of an extract of fertile soil, made with five times its weight of distilled water, was added. After a time the plants in each of the ten pots regained their green colour, and grew vigorously; whilst in only two of the thirty pots without the addition of the micro-organisms did the plants develop favourably, all the rest showing more or less nitrogen-hunger, and some were quite yellow. After a time the plants from two of the pots with bacteria, and from five without, were taken up, and examination showed very strikingly the connection between the amount of above-ground growth, and the development of the root nodules. In the 8 remaining pots with bacteria, the growth was very uniform; whilst in only 4 of the remaining 25 without bacteria was there fair development.

Hellriegel states that the quantity of soil extract added contained only 1 millig. nitrogen. In two other experiments everything was sterilised. The peas germinated healthily, developed their first 6 leaves, but did not go further, and died, not a trace of nodules being found on their roots. He concludes:—

To the nourishment of the Papilionaceæ, especially their assimilation of nitrogen, the so-called leguminous nodules, and the micro-organisms they contain, stand in close and active relation.

It was remarkable, however, that in numerous trials with lupins, under exactly similar conditions, successful second growth could not be obtained. The conclusion was, that the organisms in the root nodules of lupins were of another kind, which were less generally distributed than those found in the nodules of peas. Further, it is known that lupins do not grow well on a heavy, or even on a rich humus soil.

Three rows of the experimental pots were filled with quartz sand, a nitrogen-free solution was added, and lupins were sown. A portion of diluvial sandy soil, where lupins were growing well, was treated with 5 times its weight of distilled water, and a turbid extract was obtained, in which it might be assumed that there would be a sufficient quantity of the micro-organisms peculiar to the sandy soil. To each experimental pot of the first row, 25 c.c. of this fluid was added; those of the second row were left without any such addition; and those of the third row received the extract from the loamy humus soil, the same as was used in the case of the experiments with the peas.

The germination and early growth were favourable in all cases; then followed the hunger-condition, and after 30 days from the sowing, all the rows showed equal poverty. Then the lupins of the first row began to show fresh green colour, assumed

a healthy aspect, and grew well. The plants of both the second and third rows maintained a sickly, brown-red colour.

The roots of the plants of the first row, which received the sandy-soil extract, were thick with large root-nodules, such as are found in the field with normal vegetation. On the roots of the second series, without any soil extract, no trace of the nodules could be found; and on the roots of the third series, which received the extract from the rich soil, on only one plant was a single weak nodule observed.

Other sandy-soil Papilionaceæ, such as serradella (*Ornithopus sativa*), behaved as the lupins; whilst peas, vetches, and beans, grew best in the third row, and red clover gave no special result.

Hellriegel observes that, although the Papilionaceæ have the property of turning to account atmospheric nitrogen, they nevertheless do take up nitrogen from the soil, especially as nitrates; but he considers it doubtful whether such plants can attain to a normal development with nitrates alone, and with the exclusion of micro-organisms.

Finally, he admits that his observations require control and perfecting in various directions; and he confines himself for the present, to the simple statement of his experimental results.

In reference to the foregoing results of Hellriegel, we have already said that we have not been able to find any record of the experimental details; and indeed it seems doubtful whether determinations of nitrogen were made, either in soils, seed, or products of growth.* Nevertheless, such particulars as are given, can leave no doubt whatever that the products of growth, both of the peas and of the lupins, where favourable conditions were provided, would contain very much more nitrogen than the seed sown. If, therefore, the washed sand, and the nutritive solutions, were free from combined nitrogen, and the conditions of exposure of the experimental pots in free air were such as to exclude the possibility of the access of accidental sources of combined nitrogen, the obvious explanation is that the nitrogen gained had its source in the free nitrogen of the air.

Then, as to the conditions under which the free nitrogen has been brought into combination? The negative result with the Gramineæ, the negative result with the peas when everything was sterilised, or when the sand was not seeded by the soil-extract, the positive result with the peas when the sand was seeded by the humus soil extract, the negative result with the lupins when their soils were not seeded, or when they were seeded with the same extract as the peas, and the positive result when seeded with the extract from the sandy soil where lupins were growing, seem to exclude any other conclusion than that the micro-organisms supplied by the soil extracts were essential agents in the process of fixation. Further, the development of nodules on the roots was, to say the least, a coincident of the fixation. To Hellriegel's conclusions on this point, the objections have been raised,—first that the nodules are a result and not a cause of active growth, and that in fact they con-

* See foot-note, p. 64.

stitute a supply of reserve material for growth; and secondly that the investigations of TSCHIRCH and BRUNCHORST, prove that the nodules have no external communication with the soil. As to the latter objection, it may be observed, that Professor MARSHALL WARD has recently shown that, on the death of the nodules, the micro-organisms become distributed within the soil; and further, that in the case of HELLRIEGEL'S experiments it was the organisms themselves, or their germs, that he supplied to the soil.

It must be admitted that HELLRIEGEL'S results, taken together with those of BERTHELOT and others, do suggest the possibility that although the higher plants may not possess the power of directly fixing the free nitrogen of the air, lower organisms, which abound within the soil, may have that power, and may thus bring free nitrogen into a state of combination within the soil in which it is available to the higher plants —at any rate to members of the Papilionaceous family. At the same time it will be granted, that further confirmation is essential, before such a conclusion can be accepted as fully established.

Since the above was written, Dr. WILFARTH, who was associated with Professor HELLRIEGEL in the experiments which have been described, has given an account of a subsequent series of experiments which were made in 1887. Under the title of "Ueber Stickstoffaufnahme der Pflanzen," he gave the results at the meeting of the *Naturforscher-Versammlung*, at Wiesbaden, in September, 1887, and a short account of them was published in the 'Tageblatt,' pp. 362–63, and also in 'Versuchs-Stationen,' vol. 34, 1887, pp. 460–61, of which the following is a pretty full summary:—

He states that the previous experiments had shown, that the Gramineæ, the Chenopodiaceæ, the Polygoneæ, and the Cruciferæ, take their nitrogen from the soil, and that their growth was proportional to the available supply of nitrogen. The Papilionaceæ on the other hand take their nitrogen from the air, and grow quite normally in an absolutely nitrogen-free soil, provided a very small quantity of cultivated soil be added. He further states that they have now repeated the experiments in the same way, and that the results fully confirm the conclusions before arrived at as above stated.

The new experiments were made with oats, buckwheat, rape, peas, serradella, and lupins. The experimental soil was a pure sand, entirely free from nitrogen. Each pot contained 4 kilog. of this sand, to which were added the necessary mineral constituents. All the plants grew until the nitrogen of the seed was used up. Then to each pot a small quantity of the turbid watery extract of a surface soil was added, the quantity representing 5 c.c. of the soil, and containing from 0·3 to 0·7 millig. nitrogen. After this the different plants exhibited very great differences in growth. Neither the oats, rape, nor buckwheat showed any effect from the addition of the soil-extract, but remained in the condition of "nitrogen-hunger." On the other hand, the Papilionaceæ after a time recovered from their nitrogen-hunger, suddenly became dark green, and then grew luxuriantly up to ripeness. In experiments in which the soil-extract was sterilised by boiling, there was no such result. Peas grew well under

the influence of extract from any cultivated soil, but lupins and serradella only when extract from a soil where these plants were growing was used. The series comprised 178 pots, and the results were so accordant, as was shown by photographs exhibited, that the possibility of accident, or nitrogenous impurities, was out of the question.

Thus, it may be considered established that the Papilionaceæ can take the whole of their nitrogen from the air.

The experiments of the preceding year had shown that the peas did not derive their nitrogen from the small quantity of combined nitrogen in the air, and new experiments fully confirmed this. Following the plan of Boussingault, they put 4 kilog. of ignited sand in a large glass balloon, added mineral constituents and a small quantity of the soil-extract, and then sowed one seed of oats, one of buckwheat, and one of peas. The vessel was then perfectly closed by a well-ground glass stopper; but carbonic acid was occasionally supplied. The oats and buckwheat only grew so long as the supply of nitrogen of the seed lasted; but the peas continued to grow luxuriantly and quite normally. A large part of the produce was found to contain 6·55 grams dry substance, and 0·137 gram nitrogen.

The author says that it cannot yet be with certainty explained in what way the soil-extract enables the Papilionaceæ to assimilate the nitrogen, and that it is even doubtful whether the root-nodules have any connection with the taking up of the nitrogen. It is, however, proved that the soil-extract favours the development of the nodules, whilst the sterilised extract has no such effect. It seems natural to attribute the action to bacteria, and to connect it with the organisms in the nodules, but the experiments do not as yet settle the question.

The amount of nitrogen in the seed is not given, but to show how considerable the assimilation of nitrogen may be, the following results, showing the amounts of dry substance, and of nitrogen, in the produce of a number of the pots of lupins, are quoted :—

	Without soil-extract.			With soil-extract.	
	Dry matter.	Nitrogen.		Dry matter.	Nitrogen.
Nos.	grams.	grams.	Nos.	grams.	grams.
9	0·918	0·0146	3	44·73	1·099
10	0·800	0·0136	4	45·62	1·156
11	0·921	0·0132	5	44·48	1·194
12	1·021	0·0133	6	42·45	1·337

Such is the brief account of the experiments as yet published by Dr. Wilfarth; and that full confidence was placed in the results by those present may be inferred, since the report states that the communication was received with great applause; whilst in the discussion which followed, Drs. Nobbe, Heiden, Liebscher, Fleischer, and Emil von Wolff, took part.

It will be seen that the results are not only confirmatory of those given by HELLRIEGEL the year before, but that they are even much more definite and striking. Thus, taking no account of the fraction of a milligram of combined nitrogen supplied in the soil-extract, the amount of dry matter produced is nearly 50 times, and the amount of nitrogen assimilated is nearly 100 times, as much with, as without, the soil-extract!

The negative result with Gramineæ, Chenopodiaceæ, Cruciferæ, and Polygoneæ, is certainly just what would be expected from all that is known of the influence of soil-supplies of nitrogen on the growth of the agricultural representatives of those families. It will be observed, however, that whilst with oats and buckwheat as representatives of the Gramineæ and the Polygoneæ, HELLRIEGEL and WILFARTH got negative results, it was chiefly with rye-grass and buckwheat that M. JOULIE obtained such great gains, though it is true under very different conditions as to soil-supplies of nitrogen, whilst some of his greater gains were largely in the soil as well as in the plants.

But whilst experience, whether practical or experimental, does not point to an unsolved problem in the matter of the sources of the nitrogen of the agricultural plants of the families above enumerated, it is far otherwise so far as the Papilionaceæ are concerned. It is true that, besides other evidence, our own results, recorded in this and former papers, show that even these plants do avail themselves of nitrogen existing as nitrates within the soil; and HELLRIEGEL also distinctly recognises that such is the case. At the same time, in reference to our own experiments we have admitted that the evidence adduced does not justify the conclusion that nitrates within the soil were an adequate source of the whole of the nitrogen that was taken up in some of the cases cited. Indeed, although the question of the sources of the nitrogen of the Leguminosæ has been the subject of experiment and of controversy for about half a century, it is generally admitted that all the evidence that has been acquired on lines of inquiry until recently followed have failed to solve the problem conclusively. It should not, therefore, excite surprise that any new light should come from a new line of inquiry. Hence should be recognised, whether as real advance in knowledge, or as only incentive to further investigation, the importance of the cumulative evidence of the last few years—of which that furnished by the experiments of HELLRIEGEL and WILFARTH is certainly the most definite and the most striking, pointing to the conclusion that although chlorophyllous plants may not directly utilise the free nitrogen of the air, some of them, at any rate, may acquire nitrogen brought into combination under the influence of lower organisms, the development of which is, apparently, in some cases a coincident of the growth of the higher plant whose nutrition they are to serve.

Such a conclusion is, however, of such fundamental, and of such far-reaching importance, that further proof must yet be demanded, before it can be accepted as beyond question. Should it be eventually fully established, it would certainly suffice

to explain facts hitherto not fully explained. On the other hand, should it not be established, and a soil source of the whole of the nitrogen of the Leguminosæ be conclusively proved, the facts of agricultural production would, it seems to us, be equally well explained. To this point we shall refer again in our general concluding observations.

7. *The Experiments of Professor* Emil von Wolff.

It was also at the Berlin meeting in 1886, at which one of ourselves was present, that Professor von Wolff distributed a page of tabulated results of vegetation experiments, made at Hohenheim, and gave some account of them. A preliminary series had been made in 1883, and more careful series were conducted in 1884, 1885, and 1886. Three sets of experiments, A., B., and C., were made, as follows:—

A. In wooden boxes, 14·5 cm. (= 5·7 inches) diameter, and 28 cm. (= 11·0 inches) deep. Into each was put 8 kilog. (= 17·6 lbs.) of calcareous coarse-grained river-sand, from which the finest part had been removed by washing. The experimental plants were—oats, sand-peas, field-beans, and red clover; and each of these was grown under the following conditions:—

1.—Without manure.

2.—With mineral manure, comprising—superphosphate, magnesium sulphate, calcium carbonate, and potassium bicarbonate.

3.—With the mineral manure, and potassium nitrate = 0·208 gram nitrogen.

4.—With the mineral manure, and potassium nitrate = 0·832 gram nitrogen.

B. In sheet zinc vessels, 25 cm. (= 9·8 inches) diameter, and 35 cm. (= 13·8 inches) deep; into each of which was put 24 kilog. (= 52·9 lbs.) of the washed river-sand. These experiments were only made in 1885 and 1886. The plants were oats, and sand-peas; and the same four conditions as to manuring, as above described, were adopted; but the quantities used were larger, the amount of nitrogen supplied in Experiment 3 being 0·416 gram, and Experiment 4—1·664 gram.

C. In cement vessels, 50 cm. (= 19·7 inches) diameter and 60 cm. (= 23·6 inches) deep. Into each was put 210 kilog. (= 463 lbs.), of the raw unwashed river-sand. The conditions of manuring were in kind the same as in A. and B.; but the quantities of nitrogen supplied were, in Experiment 3—0·832 gram, and in Experiment 4—3·328 grams. These experiments were made in 1884, 1885, and 1886; and the plants were—oats, field-beans, clover, and potatoes.

At the Berlin meeting in 1886, Professor von Wolff gave only the amounts of air-dried above-ground produce; but he has since published, in conjunction with Dr. C. Kreuzhage, a long paper, giving a great deal of analytical detail.* It is there explained that, for the experiments in wooden boxes (A.), and in zinc vessels (B.), fresh washed river-sand was put in each year; but that, for those in the cement vessels (C.), the unwashed river-sand first put in was not renewed.

* "Vegetationsversuche in Sandkultur über das Verhalten verschiedener Pflanzen gegen die Zufuhr von Salpeterstickstoff." 'Landwirthschaftliche Jahrbücher,' vol. 16, Heft 4, 1887, pp. 659 *et seq.*

As to the results, WOLFF called attention to the fact that with the oats and the potatoes comparatively little increase was obtained by the use of mineral manure without nitrogen, but that where nitrogenous manure was added the increase bore a direct relation to the amount of nitric-nitrogen supplied. The behaviour of the Leguminosæ was, however, quite different. With these plants, the mineral manures as a rule gave considerable increase, whilst the addition of the nitrate generally gave little or no further increase. He remarks that these results are consistent with those obtained in ordinary agriculture; and that it is a question whether the so-called "nitrogen collectors" obtain all their nitrogen by means of their widely and deeply penetrating roots, or whether they draw some of it from the air; and if so, whether they can only take it as combined nitrogen, or also as free nitrogen? He considers that the results of BOUSSINGAULT and ourselves are against the supposition that they assimilate free nitrogen. At the same time he thinks the results of HELLRIEGEL show that the Papilionaceæ are not dependent on soil sources of nitrogen alone; though further evidence is required to determine whether or not the free nitrogen of the air comes into play.

As to the connection of the root-nodules with the development of the plants, WOLFF considers that they may be equally well supposed to be a consequence as a cause of active growth. Observations by SCHULTZ-LUPITZ, and at Hohenheim, have shown that the nodules may be very little developed in a soil rich in nitrogen. He refers to the results of FRANK and BRUNCHORST, as indicating, that the contents of the nodules do not consist either of bacteria or of fungoid forms,* but rather of nitrogenous matters which are re-absorbed by the plant when forming fruit.

As bearing on the subject, he quotes the following results of TROSCHKE at Regenwalde, showing the comparative composition of the nodules and of the roots of blue lupins, at the time of pod formation:—

	Pure ash.	Crude fat.	Crude fibre.	Crude protein.	Non-nitrogenous extract.	Nitrogen.	Of total nitrogen albuminoid.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Nodules . . .	7·51	5·33	9·43	45·31	32·42	7·25	69·7
Roots	4·07	1·31	52·25	7·06	34·61	1·13	73·7

At Hohenheim, the nodules of yellow lupins were found to be less nitrogenous at the conclusion of pod-formation.

WOLFF considers that numerous results show that atmospheric sources of nitrogen do come into play in the growth of the Papilionaceæ. He quotes from BOUSSINGAULT in reference to experiments with clover and peas grown in a nitrogen-free soil that

* It may be observed that the recent results of Professor MARSHALL WARD ('Phil. Trans.,' B, vol. 178, 1887, pp. 539–562) are at variance with the views of FRANK, BRUNCHORST, and TSCHIRCH on this point.

the plants had gained nitrogen. It is to be observed, however, that the results in question were those of BOUSSINGAULT'S earliest experiments, made in 1837 and 1838, whilst his later results with Leguminosæ obtained under similar conditions, either show slight loss or scarcely appreciable gain. WOLFF also quotes experiments of his own with clover, made in 1853, in one case in good soil, and in another in the same soil previously ignited, when the dry substance of 4 cuttings was, from the natural soil 3·396 grams, but from the ignited soil 20·314 grams; these quantities being exclusive of stubble and roots.

Referring to his more recent experiments, considerable detail is given as to the preliminary series made in 1883, as well as to the more complete series conducted in 1884, 1885, and 1886, the general conditions of which have been described above, and we now confine attention to some further account of the results obtained in the later years.

WOLFF states that the washing of the river-sand removed 1·46 per cent. of it, which consisted of sandy clayey matter. He gives a complete mineral analysis of both the crude sand, and the separated fine matter. He states that the washed sand contained only traces of nitrogen. The separated fine matter contained, however, according to a direct determination, 0·304 per cent. of nitrogen, which seemed to exist in humus compounds. It may be observed, that it was, therefore, about twice as rich in nitrogen as most ordinary arable surface soils. The results obtained in the first year 1884, in the cement vessels, with the unwashed river-sand, show the influence of this supply; but it was concluded that in 1885, and 1886, but little remained in a condition available to the plants.

All the experiments were equally exposed to the influences of air and light, excepting that the wooden boxes were placed on a low truck, which was pushed under shelter when there was violent or continued rain. All the plants were watered with distilled water when there was not sufficient rain; and in all cases the drainage was collected and used for re-watering. The sowing of the seeds was always at the end of April; the gathering of the oats, beans, and lupins, at the end of July; and the last cuttings of the clover in August and September.

To the amounts of above-ground produce (air dried), WOLFF now adds the quantity of roots, and the total weight including them. He also gives, for the produce of 1886, the amounts of dry matter, and both the percentages of nitrogen in the dry matter, and the actual quantities of nitrogen in the crops, in the case of the "C" series of experiments, that is, those made in the cement vessels, with the unwashed river-sand. In the case of the sand-peas, however, the results of the "B" series, that is, those made in zinc vessels, with the washed river-sand, are given. The following is a summary of the results, so far as the nitrogen is concerned:—

Total Nitrogen in the Crops of 1886.

	"C" series, unwashed sand.				"B" series, washed sand.
	Oats.	Lupins.	Field-beans.	Red clover.	Sand-peas.
	grams.	grams.	grams.	grams.	grams.
1. Unmanured	0·371	2·797	2·478	3·396	0·488
2. Mineral manure	0·388	9·572	4·689	8·352	2·389
3. Mineral manure, and 0·832 gram N .	1·136	5·392	3·232	8·803	3·698
4. Mineral manure, and 3·328 grams N	3·486	8·816	4·104	9·403	5·069

It should be observed that the quantities of nitrogen in manure, as stated in the table, relate to the "C" Series in unwashed sand, but the quantities supplied to the smaller vessels of the "B" Series for the sand-peas were for Experiment 3, 0·416 gram, and for Experiment 4, 1·664 gram. On the results, WOLFF remarks that without exception the cereal crop (oats) only flourished when there was a sufficient quantity of nitrogenous nutriment (nitric-nitrogen) available; whilst the Leguminosæ for the most part grew quite as luxuriantly without, or with very little, nitrogen in the soil, provided the ash constituents were in abundance. Potatoes, on the other hand, like the oats, required combined nitrogen to be provided within the soil.

The mineral composition of the crops, and the influence of the varying climatal conditions of the three seasons, are discussed in some detail. Estimates are given of the amounts of phosphoric acid, potash, &c., in the crops, calculated per hectare; and the quantity of nitrogen in the oat crop is estimated to correspond, in one case, to 139·4 kilog. per hectare (= 124·5 lbs. per acre), which is very large.

Excluding the experiments in the unwashed sand, the mean of 7 experiments under each condition of manuring with oats, and of 22 under each condition with different Leguminosæ, shows the following amounts of air-dried produce :—

	Oats. Mean of 7 experiments in each case.	Leguminosæ. Mean of 22 experiments in each case.
	grams.	grams.
1. Unmanured	16·87	22·71
2. Mineral manure	18·31	68·53
3. Mineral manure, and 0·238 gram nitrogen for the oats, and 0·180 gram for the Leguminosæ	47·42	73·39
4. Mineral manure, and 0·952 gram nitrogen for the oats, and 0·72 gram for the Leguminosæ	110·74	74·95

The little influence of the mineral, and the great influence of the nitrogenous manure, on the oats, and again the marked influence of the mineral, and the little effect of the nitrogenous manure, on the Leguminosæ, are here very strikingly illustrated.

Referring to the amounts of nitrogen in the crops of sand-peas grown in the washed sand, as given in the table at the top of page 74, WOLFF states that the 19 peas sown contained 1·542 gram dry substance, and he estimates the amount of nitrogen in the seed sown at only 0·0647 gram; the quantity of nitrogen so provided was, therefore, quite immaterial in proportion to that in the crops.

The amounts of nitrogen in the clover crops of Experiments 2 and 4 are estimated to correspond to 334 and 376 kilog. per hectare, to which one-third should be added for stubble and roots. The beans appear not to have collected so large a quantity of nitrogen in 1886 as the clover and the lupins; and it may be mentioned that whilst the 54 bean seeds sown would contain 1·0353 gram of nitrogen, the same number of lupin seeds would contain only 0·5912 gram.

Some experiments by STRECKER with yellow lupins are quoted as showing considerable gains of nitrogen.

With regard to the numerous results recorded in his paper, WOLFF admits that they are not satisfactory in all respects, those of different years not being always accordant. But the special object was to compare the growth of cereals with that of Papilionaceæ, over a series of years, in a soil poor in combined nitrogen, or containing known amounts of it; and the influence of the various seasons was different on the different crops.

The following table gives estimates of the amounts of nitrogen in the crops, more (+) or less (−) than supplied in the seed and manure, calculated per hectare; the selection of experiments being the same as in the case of the results given in the table at the top of p. 74 :—

Estimated Nitrogen, in kilograms per hectare, in crops more (+) or less (−) than in seed and manure.

	"C" series, unwashed sand.				"B" series washed sand
	Oats.	Lupins.	Field-beans.	Red clover.	Sand-peas.
	Kilog.	Kilog.	Kilog.	Kilog.	Kilog.
1. Unmanured	− 9·0	+ 88·3	+ 57·7	+ 135·8	+ 84·7
2. Mineral manure	− 8·3	+ 359·3	+ 146·2	+ 334·1	+ 464·9
3. Mineral manure, and nitrate. . .	− 11·7	+ 158·8	+ 54·6	+ 318·8	+ 643·5
4. Mineral manure, and nitrate . . .	− 17·6	+ 195·8	− 10·4*	+ 242·9	+ 668·1

* Given in error as + 89·5 by WOLFF, the nitrogen of the manure of Experiment 3 instead of that of Experiment 4 having been deducted.

It is added that these amounts do not include the nitrogen in the stubble and roots, which in the case of the oats would be very little, in that of the beans and lupins not much, but in the sand-peas considerable, as also in the clover. It is to be observed, moreover, that no account of the nitrogen in the unwashed sand is here taken. But Wolff points out that the gain was the largest in the case of the peas grown in the washed sand, which showed only a trace of combined nitrogen. Again the cement cases and their contents were exposed to the weather from year to year whilst there was no crop; but Wolff points out, as is doubtless true, that the amount of combined nitrogen brought down in the rain and dew would be quite immaterial.

Admitting it to be established that plants do not assimilate the free nitrogen of the air, he thinks the only remaining hypothesis is that certain plants are enabled to appropriate the combined nitrogen of the air, either directly through their leaves, or by absorption in the soil; and the latter he considers by far the most probable. In reference to this point he refers to the results of experiments made to determine the amount of ammonia absorbed from the air by dilute acids exposed in shallow vessels. In this way A. Müller estimated that 12 kilog. of ammonia were absorbed per hectare (= 10·7 lbs. per acre), per annum, in Sweden; whilst O. Kellner's estimate in Japan, was 14 kilog. per hectare (= 12·5 lbs. per acre). O. Kellner also determined the amounts of nitric and nitrous acids absorbed by solutions of potassium carbonate. The quantities of nitrogen so absorbed corresponded to 11·78 kilog. per hectare as ammonia, and to 1·30 kilog. as nitric and nitrous acids; giving a total of 13·08 kilog. of nitrogen per hectare (= 11·68 lbs. per acre), per annum. Other experimenters have, however, found much more. Thus, reckoning according to Schlœsing's experiments, in one of which a quantity of soil gained at the rate of 2·59 kilog. ammonia per hectare in 14 days, and in another 4·097 kilog. in 28 days, the amounts absorbed would be in the one case 68, and in the other 53 kilog. per hectare (= 60·7 and 47·3 lbs. per acre) per annum. Wolff points out that even these amounts are small compared with the quantities of nitrogen assimilated in the experimental crops. He further remarks that his porous sand would probably present more than a hundred times the absorbing surface of an acid or alkaline solution of the same area.

Besides ammonia absorption, he thinks there is probably another way in which a humus-free soil may become a source of nitrogen to plants; viz., by the combination of free nitrogen, under the influence of calcium carbonate. He quotes B. Frank ('Landw. Presse,' March 2, 1887), as having shown that a marl rich in lime, with which Schultz-Lupitz marled his soil, constantly yielded nitric acid after being boiled out with water, by which it is supposed it would be sterilised; and this was the case when the mass was exposed to ammonia-free air. According to the results obtained, a kilogram of the mass would acquire nearly a gram of nitric acid per annum. Pure calcium carbonate acted in a similar manner. After being washed out with hot water on a large filter, and kept moist, but protected from dust, nitrification took place. Frank considers the calcium carbonate of the soil as a nitrogen combiner; and that,

in presence of water and atmospheric air, nitrite and nitrate of lime are gradually formed, the nitrogen and oxygen of the air uniting in contact with the porous body, and the acid uniting with the lime and expelling carbonic acid.*

Wolff further quotes the observations of Cloëz, made more than 30 years ago, in which he passed air, first through a solution of potassium carbonate, then through sulphuric acid and over pumice moistened with sulphuric acid, for six months over various porous substances. He found a formation of nitric acid when the air was passed over pieces of brick or pumice moistened with potassium carbonate; and also traces with chalk, chalk marl, and a mixture of kaolin and calcium carbonate. On the other hand he found no formation of nitric acid by burnt bones moistened with potassium carbonate, or by clay.

Wolff considers that the conditions of his experiments involved those found by Cloëz to favour such formation of nitric acid. He admits, however, that it is difficult to explain why the action should take place when the Leguminosæ are present, and that the growth of the cereals is not benefited thereby. He suggests whether the greater pumping action of the leaves of the Leguminosæ causes a more active aëration of the soil, and so it may be that with their increased development the greater is the amount of nitrogenons nutriment accumulated from the atmosphere by the moist soil, whilst it is well known that these plants leave an efficient nitrogenous residue for succeeding crops.

In conclusion, Wolff admits that the amounts of absorption indicated in the experiments with particular plants cannot be expected on a large scale. In practice, soils are not kept so porous, and so constantly moist; nor are the mineral conditions of the soil always so favourable. Indeed the variations of result in the different experiments illustrate the influence of varying conditions.

Perhaps the most striking of Wolff's results were those obtained in the experiments made in 1853, in which clover yielded about six times as much dry produce grown in an ignited rich meadow soil, as in the same soil in its natural state. The ignited soil would not only be nitrogen free, but sterilised; so that, unless it acquired and developed micro-organisms during the growth, the supposition of the intervention of such agents in bringing free nitrogen into combination within the soil would be excluded. In reference to this point it may be remarked, that in the case of Hellriegel's experiments, in which he added the watery extract from various soils to his quartz sand, he states that red clover showed no special result.

Next as to Wolff's more recent results, in which river-sand was used as soil, in some cases unwashed, but in others washed free from the fine matter which contained nitrogen. It is, as he says, quite consistent with experience in agriculture, that oats and potatoes should yield little increase by mineral manure without nitrogen, but give increase much in proportion to the nitrogen supplied to the soil; and that, on the

* The above results of Frank have since been called in question by H. Plath ('Jahrbücher,' vol. 16, 1887, p. 891, and vol. 17, 1888, p. 725); also by Professor Landolt, 'Landw. Presse,' Jahrgang 15, no. 30.

other hand, the Papilionaceæ should give marked increase with mineral manure, and but little with nitrogenous manure. Such is certainly the result in ordinary soils containing nitrogen; but if we are to assume that in his experiments the sand was not the source of the nitrogen, both the amounts of dry produce, and those of nitrogen, in the different crops, as shown in the tables which have been given, are such as seem to exclude any other explanation than that the air had contributed nitrogen in some way.

At the same time, the conditions of experiments conducted in a not absolutely nitrogen-free soil, and with free exposure to the weather, and so subject to accidental sources of more or less combined nitrogen which such conditions necessarily imply, however appropriate for obtaining initiative results and general indications, seem scarcely suitable for the settlement of so delicate a question as that of the source of the nitrogen of vegetation. On this point it may be remarked that, according to the data given, the unwashed sand put into each cement vessel would contain about 9 grams of combined nitrogen, whilst, as shown in the table at page 74, the largest crops of the lupins and red clover, with mineral but without nitrogenous manure, only contained about that amount of nitrogen, and the beans only about half as much. It is true that this was the result in the third year, 1886, after somewhat similar amounts had already been grown for two years.

Again, so far as the sand did contain nitrogen, the great difference of result with the Gramineæ and the Leguminosæ, under the influence of mineral manure without nitrogen, is not absolutely conclusive evidence that the Leguminosæ had acquired nitrogen from some other source than the soil; for there can be little doubt that Leguminosæ do utilise nitrogen existing in the soil in a condition in which it is not available to the Gramineæ. The results obtained in the washed sand, must, however, be admitted to have much greater significance.

As to the explanation of the results, Wolff is disposed to attribute the gains of nitrogen to the absorption of combined nitrogen from the air by the soils, and to the fixation of free nitrogen within the soil under the influence of porous and alkaline bodies, as supposed by Cloëz and Frank, rather than to the fixation of free nitrogen either under the influence of micro-organisms, or directly by the plants themselves. In fact, neither were the conditions of his experiment with the clover in burnt soil, nor those of his later experiments in washed sand, such as to favour the supposition of the intervention of micro-organisms. On the other hand, he considers they were favourable for the absorption of combined nitrogen from the air, and for the supposed fixation of free nitrogen within the soil under the influence of porous and alkaline bodies. At the same time, he admits that it is not easy to explain why Gramineæ do not, equally with the Leguminosæ, benefit by such absorption, and by such fixation.

For our part we believe that a careful consideration of all that is involved in this undoubted fact, points to the exclusion of the supposition that the gain is either by the absorption of ammonia from the air, or by the fixation of free nitrogen within the

soil under the influence of porous and alkaline bodies. We shall refer to the point again in our concluding remarks, but we may here say in passing, that the results of our experiments on the growth of wheat for many years in succession on the same land without nitrogenous manure, show that with much less nitrogen annually removed in the crops, and estimated to be lost by drainage, than would be required for the growth of the Leguminosæ in WOLFF's experiments, the soil has nevertheless lost much nitrogen. Again, taking the average of ten years, the amount of nitrogen as nitric acid which has passed through 60 inches depth of soil and subsoil in the Rothamsted drain-gauges, exposed to the air and rain, with aëration from below also, but without vegetation, has been somewhat less than 40 lbs. per acre (= 44·8 kilog. per hectare) annually; and it cannot be doubted that at least the greater part of this has been derived from the organic nitrogen of the soil and subsoil. It is obvious, therefore, that the amount due to absorption and to such fixation together, must be much less than this.

If, therefore, neither nitrogenous impurity in the sand, nor accidental sources of combined nitrogen, can be supposed to account for the gains by the Leguminosæ in WOLFF's experiments, it would seem that the explanation must be sought, either in the agency of micro-organisms, or in direct assimilation by the plants themselves.

8. *The Experiments of Professor* W. O. ATWATER.

Professor ATWATER, of the Wesleyan University, Middletown, Conn., U.S.A., has published three papers in the 'American Chemical Journal':—1. "On the Acquisition of Atmospheric Nitrogen by Plants" (vol. 6, No. 6); 2 (with E. W. ROCKWOOD). "On the Loss of Nitrogen by Plants during Germination and Growth" (vol. 8, No. 5); 3. "On the Liberation of Nitrogen from its Compounds and the Acquisition of Atmospheric Nitrogen by Plants" (vol. 8, No. 5). In these papers he gives the results of experiments of his own, and discusses the results of others also, on various aspects of the question. In what years his experiments were made is not stated, but we assume in 1883 and 1884, as they were undertaken after a visit he paid to Europe in 1882, and in the autumn of 1884 he gave a paper on the subject, at the meeting of the British Association at Montreal, and also at the meeting of the American Association at Philadelphia.

It is stated that the question was:—"*May plants, grown under normal conditions, acquire any considerable amount of nitrogen, free or combined, from the ambient atmosphere?*" It is further stated, that after a series of trials had shown a not inconsiderable acquisition of atmospheric nitrogen, a second series was planned to verify the results of the first, and to include a collateral inquiry, namely:—

"*How is the acquisition of nitrogen from the atmosphere affected by abnormal conditions of growth, and what bearing may the results obtained have upon the interpretation of those obtained by other experimenters, and upon the general question of the assimilation of atmospheric nitrogen by plants?*"

Professor ATWATER first made two series of experiments in which he grew peas in sand, to which he supplied nutritive solutions, containing mineral matters, and known quantities of nitrogen as nitrate. The plants were grown in free air, but protected from rain and dew.

The following are the actual amounts of nitrogen supplied in seed and nutritive solution, the actual gains, and the gains per cent. on the total amounts supplied, in the first series of 3 experiments :—

Supplied . .	millig. .	103·7	120·6	95·7
Gains . . .	millig. .	+ 63·5	+ 13·2	+ 13·0
Gains . . .	per cent.	+ 61·2	+ 10·9	+ 13·6

The second series included 12 experiments, in six of which generally less, and in the other six generally much more, nitrogen was supplied in the solution than in the seed.

With the smaller quantities of total nitrogen the results were :—

Supplied .	millig. .	94·7	128·2	93·6	130·9	93·3	129·2
Gains . .	millig. .	+ 21·7	+ 30·7	+ 62·5	+ 27·2	+ 93·2	+ 81·7
Gains . .	per cent.	+ 22·9	+ 23·9	+ 66·8	+ 20·8	+ 99·9	+ 63·2

With the larger quantities of nitrogen the results were :—

Supplied . .	millig. .	169·3	199·3	170·5	194·5	135·8	161·0
Gains (or loss)	millig. .	+ 9·6	+ 1·3	− 20·9	+ 3·0	+142·0	+ 99·2
Gains (or loss)	per cent.	+ 5·6	+ 5·7	− 12·3	+ 1·5	+104·5	+ 62·0

With regard to the variations of result, it is concluded that where the gains were less, or there was loss, the conditions were abnormal, and where the conditions were normal, the gains were the higher.

Some of the results are calculated per hectare and per acre, showing very large gains compared with the amounts of nitrogen in ordinary crops in the field.

Numerous experiments were also made to determine the loss of nitrogen in the germination of peas ; five by the water-culture method, and eight in sand.

Three of the water-culture experiments were conducted in open air, when the results were :—

Nitrogen in seed .	millig. .	215·6	67·5	41·4
Nitrogen lost . .	millig. .	− 18·1	− 6·6	− 0·6
Loss	per cent.	− 8·4	− 9·8	− 1·5

In two water-culture experiments in the air of the laboratory, there was gain not loss of nitrogen, which was attributed to ammonia in the air. The results were :—

In seed . .	millig. .	290·2	119·7
Gains . . .	millig. .	+ 19·2	+ 10·2
Gains . . .	per cent.	+ 6 6	+ 8·5

Of the 8 germination experiments in sand, 3 were conducted in the open air. The results were :—

In seed . .	millig. .	100·3	102·9	109·7
Loss . . .	millig. .	— 6·0	— 10·9	— 16·6
Loss . . .	per cent.	— 5·9	— 10·6	— 15·1

Three experiments in a greenhouse showed :—

In seed . .	millig. .	115·6	103·4	101·4
Loss . . .	millig. .	— 8·4	— 12·7	— 16·5
Loss . . .	per cent.	— 7·3	— 12·4	— 16·3

Lastly, two experiments in a room in a dwelling-house, showed :—

In seed . .	millig. .	88·3	88·2
Loss . . .	millig. .	— 7·2	— 9·7
Loss . . .	per cent.	— 8·2	— 11·0

Atwater discusses the results of other experimenters, including those of Boussingault, Schloesing, and ourselves and the late Dr. Pugh, on the evolution of free nitrogen under various conditions. On the whole he concludes, that germination without the liberation of nitrogen is the normal process; that losses, whether during germination, or in later periods of growth, are due to forms of decay; and that they would thus be not essential to germination and growth, but accessory phenomena. He, nevertheless, gives a table showing, for 8 of his experiments, the actual gains found, the greater gains supposing there had been a loss of 15 per cent. of nitrogen—as in some of his own germination experiments, and the still greater gains supposing a loss of 45 per cent., as shown in experiments of Boussingault in the germination of beans and maize, under special conditions, in contact with nitrate. He admits, however, that there is no evidence to show what was the loss in his own vegetation experiments.

We may here remark, that, in full recognition of the loss of nitrogen under conditions of decay, it was concluded, both by Boussingault and at Rothamsted, that excepting in some cases in which there was obviously some decay, the results were not vitiated by any such loss, and in those cases the losses found were so explained.

Atwater quotes Boussingault's earliest experiments, made in 1837 and 1838, to show that with clover and peas nitrogen must have been gained from the air. It

should be remarked, however, that BOUSSINGAULT himself, after he had acquired much more experience, both in the conduct of vegetation experiments, and in analytical method, entirely disallowed that the results of those early experiments were evidence of the fixation of free nitrogen. He also quotes BOUSSINGAULT'S later experiments; made in 1851, 1853, and 1854, and says in regard to them that—"even the cereals, oats and wheat, contain 17 to 32 per cent. more than was supplied in the seed. The results differ from those of the previous series in that the cereals here show more, and the legumes less, gain of nitrogen." Now, in the 6 cases with haricots and lupins referred to, the amounts of nitrogen supplied in the seed ranged from 19·9 to 36·7 millig., and they show, respectively—2·3 millig. actual loss, and 3·2, 2·5, 4·2, 3·0 and 2·0 millig. actual gain. In the case of the cereals, on the other hand, the total amount of nitrogen supplied in the seed was, with the oats only 3·1, and with the wheat only 6·4 millig., and the actual gains shown were only 1·0 and 1·1 millig. Yet, ATWATER shows that these last results, calculated into *percentage*, represent gains of 32 and 17 per cent. of the original nitrogen, and larger gains even than with the legumes! It need hardly be said, that BOUSSINGAULT interpreted these later results as not indicating any fixation of nitrogen.

Briefly summarised, ATWATER'S conclusions are:—

1.—That in some of his experiments with peas, half or more of the total nitrogen of the plants was acquired from the air. Where the gains were small, or there was a loss, the conditions were abnormal, and it is to be assumed that there was loss, either from the nitrate of the nutritive solutions, from the seeds during germination, or from the growing plants.

2.—An actually observed gain is positive proof that nitrogen has been assimilated, either directly by the plants, or indirectly through the medium in which the roots have developed. The failure of an experiment to show gain only proves non-assimilation, if it is also proved that there was no liberation of nitrogen. The conflicting results of various experimenters may probably be explained by the fact of such liberation.

3.—The experiments do not show in what way the nitrogen is acquired. It must have been taken up, either as free or combined nitrogen, either directly through the foliage, or indirectly through the soil and nutritive solutions, and the roots.

4.—It is possible that the negative results of BOUSSINGAULT, and ourselves, are due to the liberation of free nitrogen. The conditions were, moreover, such as to exclude the action of electricity and of microbes.

5.—Since BERTHELOT has shown that nitrogen may be fixed in organic matter by the agency of electricity, and in soils by the agency of micro-organisms, some cases of gain may be so explained; but it is considered that the conditions for such actions did not exist in his own experiments. The balance of evidence favours the assumption that the plants themselves were the agents.

6.—The conclusion that plants acquire atmospheric nitrogen accords with, and

explains, facts of vegetable production otherwise unexplained; and the fact of its acquisition in considerable quantities seems well established.

We have pointed out that in BERTHELOT's later papers he seems to rely much more on the agency of micro-organisms, than on that of electricity, in explanation of the phenomena of the fixation of free nitrogen; whilst ATWATER does not consider that the conditions of his own experiments are favourable to the supposition that either of these agencies was the cause of the fixation which his results show.

It need only be added, that the assumption that the real gains are generally greater than the experimental results indicate, on account of the losses that have taken place, is a very old one, it having been brought against the negative results obtained by BOUSSINGAULT, and at Rothamsted, thirty or more years ago. It is still, however, as has been seen, a favourite argument with others as well as Professor ATWATER. We have already said, that neither the conclusions of BOUSSINGAULT, nor those drawn from the Rothamsted experiments, were vitiated by virtue of such loss. Further, the supposition that the assimilation of free nitrogen is the greater when luxuriance is favoured to a certain degree by artificial supplies of combined nitrogen, owes its origin to about the same date. The result was, however, as distinctly negative in the experiments at Rothamsted when luxuriance was so favoured, as when it was not. It is freely admitted, however, that in many of the experiments of BOUSSINGAULT, as in those at Rothamsted, the arrangements were such as to exclude the agency, either of electricity or of micro-organisms. To this point we shall refer again presently.

9. *Recent results and conclusions of* M. BOUSSINGAULT.

We have frequently discussed the results of M. BOUSSINGAULT, obtained from 1837 to 1854, and expressed entire agreement with the conclusions he drew from them under the conditions provided; and it is not the object of the present comments to reconsider them in any detail. The question of the fixation of free nitrogen has, however, assumed a new aspect in recent years. It is now supposed that fixation, either by the plant, or within the soil, takes place, if at all, by the agency of electricity, or of micro-organisms, or of both; and there can be no doubt, that the earlier vegetation experiments of BOUSSINGAULT above referred to, as well as those conducted at Rothamsted about thirty years ago, were so arranged as to exclude the influence of either of those agencies. If, therefore, it should be established, that fixation does take place under their influence, and that such influence is essential for the development of the action, the conclusions, both of BOUSSINGAULT and ourselves, from the results in question, are so far vitiated. It is to some of BOUSSINGAULT's more recent results, which have a bearing on this new aspect of the question, that we propose now to refer.

In BOUSSINGAULT's previous vegetation experiments he had used sterilised materials as soils. But, in 1858,* he commenced a series in which he employed more or less of a

* 'Comptes Rendus,' vol. 48, 1859, p. 303. 'Agronomie, &c.,' 2e édit., vol. 1, 1860, p. 283.

rich garden surface soil, mixed with more or less sand, or quartz, or both. The plants grown were lupins, hemp, and haricots; in some cases in free, and in others in confined air. In the latter cases, the materials were put into a large glass balloon, or carboy, moistened with pure distilled water, the seed sown, and then the whole perfectly closed from the outer air by means of caoutchouc, arrangement being made, however, for the supply of carbonic acid.

In the experiment with lupins in confined air, the largest amount of the rich soil was used, and the result was so striking, that Boussingault repeated it the next year, 1859,* when he obtained an almost identical result. In no other case was there anything like the same amount of gain of nitrogen, and we must only refer in any detail to the conditions and the results of these two experiments. They were as follows:—

Date.	Plant.	Air.	Soil, &c.				Nitrogen.						
							At commencement.			At conclusion.			
			Soil.	Sand.	Quartz.	Ash.	In soil.	In seed.	Total.	In soil.	In plant.	Total.	Gain.
			grams.	grams	grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.	grams.
1858	Lupin	Confined	130	1000	500	0·2	·3393	·0204	·3597	·4045	·0246	·4311	+·0714
1859	Lupin	Confined	130	720	150	0·1	·3380	·0200	·3580	·3834	·0417	·4251	+·0671

It should be stated that, taking the mean of 7 determinations by the soda-lime method, the rich garden soil used contained 0·261 per cent. of nitrogen. This Boussingault calculated would correspond to 11,310 kilog. nitrogen per hectare (= 10,098 lbs. per acre), one-third of a metre deep. He also determined the amounts of ammonia, nitric acid, and carbon, in the soil; and he concluded that the nitrogen, beyond the small amount existing as ammonia or nitric acid, was in combination as organic matter. In fact it existed in organic detritus, and especially in a black substance which he observed by the microscope.

Referring to the figures, it is seen that in the experiment in 1858 there was a gain of 0·0714 gram nitrogen, upon a total of 0·3597 supplied in soil and seed. Further, calculation shows that, of the total gain 0·0672 gram was in the soil, and only 0·0042 gram in the plant, notwithstanding that the original soil contained 0·3393 gram nitrogen. Boussingault remarks that the fertilising matters in the soil had thus scarcely taken any part in the growth; the conclusion being that it was only the nitrogen that existed as, or was transformed into, ammonia or nitric acid, that was available. He further remarks, that it was impossible, that anything like the amount of excess of nitrogen in the soil could be due to the débris of the vegetable matter of the lupin, roots, &c. He adds:—*It is the soil and not the plant which has fixed the nitrogen; and one such result can only be admitted if confirmed by future experiments.*

* 'Agronomie, &c.,' p. 329.

In the same year, 1858, another experiment was made with lupins, but in free air, one with hemp in free air, two with haricots, one in confined and one in free air, and one with 120 grams of the rich soil placed in a shallow vessel, kept moist with distilled water, and exposed to the free air as an experiment on fallow. In this last experiment, whilst there was a loss of about one-third of the carbon, the nitrogen increased from 0·31320 gram to 0·32184 gram, showing a gain therefore of 0·00864 gram.

Boussingault remarks that whilst the soil has lost a considerable quantity of its carbon by slow combustion, the nitrogen instead of diminishing has even increased, and that it remains to decide whether there has been nitrification, production, or simple absorption of ammonia.

In introducing the report of the second series of experiments, those made in 1859, Boussingault says that he could not accept the gain of nitrogen by vegetable mould as sufficiently established, without repeating the experiments. It further remained to examine whether, in case there really were a fixation of nitrogen, it was as nitric acid, as ammonia, or as organic compounds.

In reference to the result of the experiment made in 1859, as given in the table, Boussingault says that during the growth of a lupin in a confined atmosphere, in 130 grams of very rich soil, mixed with sand to favour the access of air, the plant, during 97 days, assimilated 0·0217 gram nitrogen from the soil, and yet the soil gained 0·0454 gram nitrogen, only one-ninth of which pre-existed as nitric acid or ammonia. The total gain of nitrogen in plant and soil was 0·0671 gram, a result which is almost identical with that found in 1858. He adds, that there is this curious coincidence, that in both cases it is by the soil, and not by the plant, that the gain has been effected.

In the case of none of the other vegetation experiments in 1859 are the gains or losses by the soils given, so that the total gain or loss cannot be estimated. Boussingault points out, however, that in 1859, there was about twice as much nitrogen taken up by a haricot growing in 100 grams of the soil, as by one growing in only 50 grams in 1858. It may be added that haricots took up much more nitrogen in proportion to a given amount of soil than lupins.

Referring to the main results, Boussingault says the singular fact appears, that the soil not only gained ammonia and nitrates, but organic matter also, possibly the remains of living organisms. On careful examination, he has observed that vegetable earth contains, not only dead organised matter, but living organisms, germs, the vitality of which is suspended by drying, and re-established under favourable conditions as to moisture and temperature. This mycodermic vegetation is not always visible to the naked eye, and its progress must be followed by the aid of the microscope. The mycoderms have only an ephemeral existence, and they leave their detritus in the soil, which in time may give rise to ammonia and nitric acid. Even if the nitrogen of the air takes part in nitrification, a part of the nitrogen will exist in mycoderms, or their remains.

However this may be, considering only the numerical results which have been obtained, he is forced to believe that the soil of Liebfrauenberg has fixed nitrogen; nitric acid and ammonia being at the same time developed. The experiment on fallow in confined air seems to indicate that the vegetation has but little to do with the result.

Having given the details of his experiments, he submits them to the criticism of others, thus enabling them to judge whether the intervention of the nitrogen of the air in the production of nitrates is really established. In his opinion, if there is not absolute proof, there is certainly strong presumption, in favour of the reality of the phenomenon.

These very remarkable results seem to have instigated new experiments to test the validity of the obvious conclusion from them. In the discussion of the previous experiments Boussingault had constantly compared the results obtained in a vegetable soil with those in a nitre bed. In reference to these new experiments he says that in the nitrification of vegetable earth, and in the materials of an artificial nitre bed, everything leads to the conclusion that the nitric acid is developed especially at the expense of organic substances. But it does not necessarily follow that the gaseous nitrogen of the atmosphere cannot contribute, within certain limits, to the production of nitrates. It is to ascertain whether this co-operation takes place that the new experiments were undertaken.

In the next year, 1860,* Boussingault placed a mixture of 100 grams of very rich vegetable soil, and 300 grams of quartz sand, in a large balloon, such as he used in the previous vegetation experiments, moistened the mass, and then closed it perfectly by means of a caoutchouc cap. A second experiment was also arranged, in which the conditions were precisely similar, excepting that 5 grams of cellulose were added to the mixture. The materials could not be stirred, and it was decided to leave them in contact with confined air for a considerable time. The two vessels were, in fact, left for 11 years, when, in 1871, they were opened, and the contents examined.

The result was that in both cases there was a very considerable amount of nitrification, representing in Experiment 1 rather more, but in Experiment 2 with the cellulose less, than one-third of the original nitrogen of the soil. The actual loss of carbon was more than 4 times as great in Experiment 2 with the cellulose, as in Experiment 1 without it; amounting in Experiment 1 to about 16 per cent., and in Experiment 2 to about 43 per cent. of the original quantity.

Lastly, as to the nitrogen:—In Experiment 1, without cellulose, there was out of 0·4722 gram total nitrogen in the original soil, a loss of 0·0212 gram, corresponding to 4·5 per cent. of the original amount; and in Experiment 2, with the cellulose, there was, upon the same original amount of nitrogen a loss of 0·0081 gram, corresponding to 1·71 per cent. of the original.

* 'Compt. Rend.,' vol. 76, 1873, p. 22.

In regard to these new results BOUSSINGAULT says that, contrary to his anticipation, the combustion of the carbon of the non-nitrogenous organic matter, the cellulose, added to the soil, had not favoured the production of nitric acid.

He gives reasons for concluding that the process of nitrification had been completed before the opening of the vessels in 1871. At the same time, he shows that the amount, both of oxygen, and of salifiable bases, remaining, was sufficient for the production of much more nitrate.

Upon the whole he concludes as follows :—

It results from these researches, that, in the nitrification of vegetable soil, in a confined atmosphere not renewed, that is in stagnant air, gaseous nitrogen does not appear to contribute to the formation of nitric acid. The nitrogen determined in the soil in 1871, was not more than, but was even not quite so much as, in 1860. In the conditions of the experiment, the nitrification must have taken place at the expense of the organic substance of humus, which is found in all fertile soils.

Although, as we have already said, the experiments in question were obviously suggested by the results obtained in 1858 and 1859, which showed a gain of nitrogen, BOUSSINGAULT does not, throughout the discussion of the new results, offer any explanation of, or even refer to, the earlier ones. Further, it will be observed that in recording the negative results of the new experiments, he is careful to define the conditions under which they were obtained.

Always placing the greatest reliance, both in the work and in the conclusions of BOUSSINGAULT, we had been much impressed with the significance and importance of the earlier results and conclusions above referred to, which did not seem to be satisfactorily explained by the new ones, and in April, 1876, one of us wrote to him as follows :—

"We have been very much struck with some of your results with Leguminosæ, especially those with lupins in confined air in 1858 and 1859, and those with lupins and haricots in free air in 1858. May I ask whether it is your opinion that the free azote of the air does enter into combination, either by the direct agency of vegetation, or through that of the soil? And, if so, under what conditions do you think this action takes place, and what is the nature of the action?"

In answer BOUSSINGAULT wrote a long and interesting letter, dated May 19, 1876, in which he discussed various points of the subject of the sources of the nitrogen of vegetation, and replied as follows in reference to the special questions relating to his experiments in 1858 and 1859 :—

"Quant à l'absorption de l'azote gazeux de l'air par la terre végétale je ne connais pas une seule observation irréprochable qui l'établisse ; non seulement la terre n'absorbe pas d'azote gazeux mais elle en émet, ainsi que vous l'avez reconnu avec Mr. LAWES, comme l'a vu REISET pour le fumier, comme nous l'avons constaté, M. SCHLŒSING et moi, dans nos recherches sur la nitrification.

"S'il est en physiologie un fait parfaitement démontré, c'est celui de la non assimilation de l'azote libre par les végétaux, et je puis ajouter par les plantes d'un ordre inférieur, telles que les mycodermes, les champignons."

Thus, then, although by the terms of our inquiry, BOUSSINGAULT'S attention was specially directed to the evidence of gain of nitrogen from the air by the soil which his experiments in 1858 and 1859 afforded, he, in 1876 states that he is not aware of any irreproachable observation establishing the reality of such an action, whilst, on the contrary, he considers it established that soils emit rather than gain free nitrogen.

Further, he considers it perfectly demonstrated, that neither plants of a higher, nor those of an inferior order, such as mycoderms and fungi, assimilate free nitrogen.

It is to be observed that, although BOUSSINGAULT clearly ignores the significance of the results to which we had directed his attention, he did not offer any explanation of them. Subsequently, on several occasions when passing through Paris, one of us sought to meet M. BOUSSINGAULT, and to discuss the question with him further, but he was each time in Alsace. However, one of us visited him at Liebfrauenberg in 1883, and had an interesting conversation with him on the subject. No special reference was made to his experiments of 1858 and 1859; but he clearly maintained the same view as to the non-fixation of free nitrogen, as given in the sentences above quoted from his letter of 1876.

It is remarkable, that in that letter he should so expressly give his opinion against the supposition that the lower organisms within the soil effect the fixation of free nitrogen, notwithstanding the evidence of his experiments of 1858 and 1859 that the gain, if there really were gain, was chiefly by the soil, and chiefly as organic matter, the accumulation of which he attributed to the development of mycodermic vegetation. It is true that, in the discussion of the results, he did not give any clear indication whether he considered that the apparent fixation was due in the first instance to the process of nitrification, the mycoderms only appropriating the nitrogen of the nitrates formed, or whether he supposed that the mycoderms themselves were the primary agents, and that the nitrification was only the result of the oxidation of the mycodermic remains.

It did indeed seem, that, in the results in question, there was *the germ of the germ explanation* of the fixation of free nitrogen, if such took place at all, in connection with vegetation. But we confess that BOUSSINGAULT'S very distinct conclusion against the assumption of any such agency, notwithstanding the indications of some of his own experiments, leads us still to ask for further confirmation of the evidence of others in the same direction, which has been accumulating during the last few years.

PART III.

Summary, and General Considerations and Conclusions.

It seems desirable to endeavour to summarise the results, both experimental and critical, of this extended inquiry, relating to a very difficult and very complicated subject, and involving the consideration of very conflicting evidence, and of equally conflicting opinions in regard to it.

We will first give a *résumé* of the results, and conclusions, as given in Part I. of this paper, on—

1. *The Evidence relating to other Sources than Free Nitrogen.*

In our earlier papers we had concluded that, excepting the small amount of combined nitrogen coming down in rain and the minor aqueous deposits from the atmosphere, the source of the nitrogen of our crops was, substantially, the stores within the soil and subsoil, whether derived from previous accumulations, or from recent supplies by manure.

More recently, we have shown that the amount of nitrogen as nitric acid in the soil, was much less after the growth of a crop than under corresponding conditions without a crop. In the case of gramineous crops it was concluded that most if not the whole of their nitrogen was taken up as nitric acid. In the experiments with leguminous crops the evidence indicated that, in some cases the whole of the nitrogen had been taken up as nitric acid, but that in others that source seemed to be inadequate.

It has been further shown that, under otherwise parallel conditions, there was very much more nitrogen as nitric acid in soils and subsoils, down to a depth of 108 inches, where leguminous than where gramineous crops had for some time been grown. The indication was, that nitrification had been more active under the influence of leguminous than of gramineous growth and crop residue.

At the same time, comparing the amounts of nitrogen as nitric acid in the soil where the shallow rooting *Trifolium repens* had previously been grown, with those where the deeper rooting *Vicia sativa* had yielded fair crops, it was found that, down to a depth of 108 inches, the *Vicia* soil contained much less nitric acid than the *Trifolium repens* soil; and it was concluded that most, if not the whole, of the nitrogen of the *Vicia* crops had been taken up as nitric acid.

New results of the same kind, which related to *Trifolium repens* as a shallow rooting and meagrely yielding plant, to *Melilotus leucantha* as a deeper rooting and freer growing one, and to *Medicago sativa* as a still deeper rooting and still freer

growing plant, very strikingly illustrated and confirmed the result of the exhaustion of the nitric acid of the subsoil by the strong, deep-rooting, and high nitrogen-yielding Leguminosæ. Still, the figures did not justify the conclusion that the whole of the large amount of nitrogen taken up by the *Medicago* crops could have had its source in nitric acid. It was obvious that much nitrification takes place near the surface; but as the surface soil became even somewhat richer in nitrogen, it was clear that it had not been the primary source of the whole of the nitrogen taken up by the plants. The source of much of it must have been either the atmosphere, or the subsoil; and if the subsoil, and yet not wholly as nitric acid, the question arises, in what other form of combination ?

In another experiment, where one leguminous crop—beans—had been sown for many years in succession, but had frequently yielded very small crops, and sometimes failed, and over the whole period had given an average of little more than 30 lbs. of nitrogen per acre per annum, the land was then left fallow for several years, after which, in 1883, barley and clover were sown. In that year, in 1884, and in 1885, about 300 lbs. of nitrogen were removed per acre, chiefly in the clover crops. This result was obtained—where another leguminous crop had to a great extent failed, where the surface soil had become very poor in total nitrogen, where there existed a very small amount of ready formed nitric acid to a considerable depth, and where the surface was unusually poor in nitrogenous crop residue for nitrification.

Further, not only had this large amount of nitrogen been removed in the clover crops, but the surface soil became determinably richer in nitrogen. Here again, then, the primary source of the nitrogen of the crop could not have been the surface soil itself. It must have been either the atmosphere, or the subsoil; and assuming it to be the subsoil, the question arises whether it was taken up as nitric acid, as ammonia, or as organic nitrogen ?

The various results adduced could leave no doubt that nitric acid was an important source of the nitrogen of the Leguminosæ. Indeed, existing evidence relating to nitric acid carries us quantitatively further than any other line of explanation. But it is admittedly inadequate to account for the amounts of nitrogen taken up, either by the *Medicago sativa* on the clover-exhausted land, or by the clover on the bean-exhausted land.

Direct experiments were made to determine whether the nitrogen of the Rothamsted raw clay subsoils, from which it was assumed much nitrogen had been derived in some way, was susceptible of nitrification, provided the nitrifying organisms, and other necessary conditions, were present. It was found that the nitrogen of such subsoils, containing only between 0·04 and 0·05 per cent. of nitrogen, and not more than six or eight parts of carbon to one of nitrogen, was susceptible of nitrification. It was also found that nitrification was more active in leguminous, than in gramineous crop subsoils.

Although it was clear that the nitrogen of raw clay subsoils, which constitutes

an enormous store of already combined nitrogen, was susceptible of nitrification, provided the organisms are present, and the supply of oxygen is sufficient, the results did not indicate that these conditions would be adequately available in such cases as those of the very large accumulations of nitrogen by the *Medicago sativa* for a number of years in succession on the clover-exhausted land, or by the red clover on the bean-exhausted land.

The question arose—whether roots, by virtue of their acid sap, might not, either directly take up, or at any rate attack and liberate for further change, the otherwise insoluble organic nitrogen of the subsoil? Accordingly, specimens of the deep, strong, fleshy root, of the *Medicago sativa* were collected and examined, when it was found that the sap was very strongly acid. The degree of acidity was determined, and attempts were made so to free the extract from nitrogen so as to render it available for determining whether or not it would attack and take up the nitrogen of the raw clay subsoil. Hitherto, however, these attempts have been unsuccessful.

When this difficulty arose, it was decided in the meantime to examine the action on soils and subsoils, of various organic acids, in solutions of a degree of acidity either approximately the same as that of the lucerne root-juice, or having a known relation to it.

It was found that the weak organic acid solutions did take up some nitrogen from the raw clay subsoil, and more from the poor lucerne surface soil. But when solutions of only approximately the acidity of the root-sap were agitated with an amount of soil which it was thought would be sufficient to yield so much nitrogen as to insure accurate determination, it was found that the acid frequently became neutralised by the bases of the soil, and that less nitrogen remained dissolved after a contact of 24 hours, or more, than after only 1 hour. The strength of the acid liquids was therefore increased, and the relation of soil to acid diminished. More nitrogen was then taken up, and more after the longer than after the shorter period of contact. Still, on adding fresh acid solution to the already once extracted soil, a limit to the amount of nitrogen rendered soluble was soon reached.

Here again, the conditions of experiment in the laboratory are not comparable with those of the action of living roots on the soil, and the results obtained did not justify any very definite conclusion as to whether the action of the roots on the soil, by virtue of their acid sap, is quantitatively an important source of the nitrogen of plants having an extended development of roots, of which the sap is strongly acid.

Dr. G. Loges has published the results of experiments in which he acted upon soils by pretty strong hydrochloric acid, and determined the amount of nitrogen taken up. One of his soils contained, however, 0·804, and the other 0·367, per cent. of nitrogen; whilst the surface soil of the lucerne plot at Rothamsted contained only about 0·125, and the subsoil, which is assumed to have yielded large quantities of nitrogen to the crops, little more than 0·04 per cent. Again, in the one case Loges found 40 per cent., and in the other 22·6 per cent., of the total nitrogen taken up.

It is obvious, therefore, that such an action is not directly comparable with that of root-sap on a poor subsoil. LOGES concluded however that the substance taken up is an amide or peptone body.

MM. BERTHELOT and ANDRÉ have also published the results of experiments to determine the character of the insoluble nitrogenous compounds in soils, and of the changes they undergo when acted upon by hydrochloric acid. They found the nitrogen in the extract existed partly as ammonia, but in much larger proportion as soluble amides, and that the amounts obtained of both, increased with the strength of the acid, the time of contact, and the temperature. They also found that when the clear filtered acid extract is exactly neutralised by potash, one portion of the amide still remains soluble, whilst another is precipitated, showing that the amides rendered soluble constitute two groups. Such re-precipitation is quite in accordance with the results obtained in our own experiments, in which less nitrogen remained dissolved after 24 hours' than after only 1 hour's contact, when, with the longer period, the acidity of the extract became neutralised.

In the experiments of BERTHELOT and ANDRÉ, as in those of LOGES, the strength of acid used was much greater than in the Rothamsted experiments, and very much greater than is likely to occur in any root-sap. Further, the soil they operated upon was about 4 times as rich in nitrogen as the Rothamsted subsoils, and with the strongest acid, and a temperature of 100° C., nearly one-third of the total nitrogen of the soil was dissolved.

Still, the results of LOGES, and of BERTHELOT and ANDRÉ, are of much interest as confirming the supposition that the insoluble nitrogenous compounds in soils are, or yield, amide bodies, and as indicating the changes to which they are subject when acted upon by acids. Supposing, however, the acid root-sap so to act on the insoluble organic nitrogen of the soil, and especially of the subsoil, the question still remains, whether the amide rendered soluble is taken up as such, or undergoes further change before serving as food for the plant? It is seen that ammonia is an essential result of the reaction; and the further question arises, therefore, whether the liberated ammonia is taken up as such, or is first oxidated into nitric acid? Then, again, is the soluble amide subjected to further change, perhaps first yielding ammonia, and this again nitric acid? On this supposition we are again met with the difficulty as to the sufficient aëration of the subsoil.

Independently of much other evidence, our own direct experiments have shown it to be probable, if not certain, that fungi can utilise both the organic carbon and the organic nitrogen of the soil; though they seem to develop the more freely when the humic matters have not undergone the final stages of change by which the compound of so low a proportion of carbon to nitrogen as is found in raw subsoils, has been produced. As bearing on the question whether amides, rendered soluble within the soil, may be taken up as such by chlorophyllous plants the results of various experiments

of others, made to determine whether such plants can take up such bodies, and assimilate their nitrogen, have been considered.

Upon the whole it seems probable, that green-leaved plants can take up soluble complex nitrogenous organic bodies, when these are presented to them under such conditions as in water-culture experiments, and that they can transform them and appropriate their nitrogen. If this be the case, it would seem not improbable that they could take up directly, and utilise, amide bodies rendered soluble within the soil by the action of their acid root-sap.

In connection with the subject of the conditions under which the insoluble organic nitrogen of soils and subsoils may become available to chlorophyllous plants, some results of FRANK are referred to. He observed that the feeding roots of certain trees were covered with a fungus, the threads of which forced themselves between the epidermal cells into the root itself, which in such cases had no hairs, but similar bodies were found external to the fungus-mantle, which prolonged into threads among the particles of soil. FRANK concluded that the chlorophyllous tree acquires its soil nutriment through the agency of the fungus.

Such a mode of accumulation by some green-leaved plants, obviously allies them in this respect very closely to fungi themselves; indeed, it is by an action on the soil which characterises non-chlorophyllous plants, that the chlorophyllous plant acquires its soil-supplies of nutriment. But inasmuch as, in the cases observed, the action was most marked in the surface layers of soil rich in humus, and it is stated that the development has not been observed on the roots of any herbaceous plants, the facts so far recorded do not aid us in the explanation of the acquirement of nitrogen by deep and strong rooted Leguminosæ from raw clay subsoils. Still, in view of the office within the soil which is by some attributed to micro-organisms, and other low forms, the observations are not without interest.

It is admitted that existing evidence on the various points which have been referred to is insufficient to explain the source of the whole of the nitrogen of the Leguminosæ.

The question arises, therefore, whether the free nitrogen of the atmosphere is fixed, either by the plant, under the influence of electricity or otherwise, or within the soil, by the agency either of electricity or of micro-organisms? We believe that the results of BOUSSINGAULT, and those obtained in conjunction with the late Dr. PUGH at Rothamsted, are conclusive against the supposition of the fixation of free nitrogen by the higher plants, under conditions in which the possibility of electrical action, or of the influence of micro-organisms, is excluded. The following is a brief *résumé* of the more detailed account and discussion, given in Part II., of the recently published results and conclusions of others, from experiments for the most part made under such conditions as not to exclude the possibility of the influence of electricity or of micro-organisms.

2. *The Evidence relating to the Fixation of Free Nitrogen.*

In the experiments of M. BERTHELOT, in all of which the gains of nitrogen are comparatively small, they have in some cases been attributed to electrical action, and in others to the agency of micro-organisms within the soil.

M. BERTHELOT first showed that free nitrogen was fixed by various organic compounds under the influence of the silent electric discharge, at the ordinary temperature; and he suggested that such actions probably take place in the air during storms, and when the atmosphere is charged with electricity, organic matters absorbing nitrogen and oxygen. He also experimented with currents of much weaker tension, more comparable with those incessantly occurring in the air, and in all cases he found that nitrogen was fixed by the organic substance. The gains were in amount such as would explain the source of the nitrogen which be considers crops must derive from the atmosphere.

Subsequently, he found that free nitrogen was brought into combination by argillaceous soils, when exposed in their natural condition, but not when they were sterilised. He also found gain when the natural soils were enclosed. He considered the results showed that there was gain of nitrogen quite independently of any absorption of combined nitrogen; in fact that there was fixation of free nitrogen due to living organisms. He further considered that such gains, not only serve as compensation for exhaustion by cropping, &c., but explain how originally sterile argillaceous soils eventually become vegetable moulds.

He also made experiments on the fixation of free nitrogen by vegetable earth supporting vegetation; and he found that there was a gain about equally divided between the soil and the plant, the latter having taken it up from the soil, which he considers is the true source of gain.

The results obtained under the influence of the silent discharge in bringing free nitrogen into combination with certain vegetable principles, of course owed their special interest to the inference that thus free nitrogen might be brought into combination within the plant; but M. BERTHELOT now considers it doubtful whether the higher plants do bring free nitrogen into combination at all. Obviously, however, if there are organic compounds within the soil which have the power of bringing free nitrogen into combination under the influence of electricity, the soil may be the source, and yet the agent may be the feeble electric current. But, so far as it is assumed that free nitrogen is brought into combination in the atmosphere itself, the resulting compounds will be found in the air, and in the aqueous depositions from it; and the limit of the amount of combined nitrogen so available over a given area, in Europe at any rate, is pretty well known.

In conclusion, although it must be admitted that M. BERTHELOT carefully considered, and endeavoured to estimate, all other sources than free nitrogen, yet the conditions of risk and exposure to accidental sources of gain, in experiments in open

air, are such that results so obtained cannot of themselves be accepted without reservation. But the fact that he found distinct gains in experiments in closed vessels, and that he obtained negative results with sterilised soils, is certainly in favour of the conclusion at which he arrived.

M. JOULIE made numerous vegetation experiments in which the soils and the plants were, with certain precautions, exposed to the free air, and in which known amounts of combined nitrogen were supplied. He found very variable, but in some cases very large, gains of nitrogen. He considered that the variations of result were largely due to the varying conditions as to mineral-supply in the different experiments.

M. JOULIE concluded that microbes probably play an important part in the fixation of nitrogen. He did not think that his results were favourable to the supposition that the plants themselves effected the fixation. For the present he limits himself to the establishment of the great fact of the fixation of the free nitrogen of the atmosphere, leaving to the future the exact explanation.

It is to be observed that the large gains shown were chiefly with a polygonous plant, buckwheat, and not with plants of the leguminous family, which are reputed to be "nitrogen collectors."

To show the practical importance of the fixation of free nitrogen, M. JOULIE calculates what would be the gain per hectare according to some of his results. It may be confidently affirmed, however, that such gains as he so estimates, do not take place, either with or without vegetation, in ordinary soils, in ordinary practice.

Dr. B. E. DIETZELL made vegetation experiments, in which plants were watered with distilled water, the drainage was returned to the soils, and the pots and their contents were exposed to free air, but protected by a linen roof; a rich garden soil, containing 0·415 per cent. of nitrogen, was used, several different conditions as to manuring were adopted, and peas and clover were the subjects of experiment. Thus the plants were of the leguminous family; but notwithstanding this, there was, in no case, a gain of nitrogen. In one there was neither gain nor loss, and in all the others there was a loss, in some cases amounting to about 15 per cent. of the total nitrogen involved.

That there should be loss with a soil containing 0·415 per cent. of nitrogen, that is about three times as much as most ordinary arable soils, is not at all surprising; and it is seen that, neither from the combined nitrogen of the atmosphere, or that due to other accidental sources, nor from free nitrogen, either directly or indirectly, did these reputed "nitrogen-collectors" gain nitrogen to compensate the losses from the rich soil. Indeed, Dr. DIETZELL's results are quite accordant with well established facts.

Professor FRANK also made vegetation experiments in free air. His soil was a humus-sand, containing only 0·0957 per cent. nitrogen; distilled water was used for watering, and the vessels were deep and narrow cylinders, without any arrangement at the bottom for drainage, or for aëration.* In three experiments without a plant, in one with two lupins, and in one with one lupin and incarnate clover together,

* See foot-note at p. 61.

there was a loss of nitrogen; whilst in one with three lupins, and in one with one lupin there was a gain. FRANK considered it probable that where a loss was indicated with vegetation, there had nevertheless been a gain, but not enough to compensate the loss.

In another experiment, with a soil about 12 times as rich in nitrogen, and many times richer than ordinary arable soils, he found a loss, due mainly to evolution of free nitrogen; and referring to this result, he says that if such losses take place in ordinary agriculture there must be natural compensation.

In the experiments in the deep and narrow vessels, without drainage, and without plants to cause evaporation, movement, and aëration, loss by evolution of free nitrogen is only what would be expected. Such loss would also be expected in the two cases of loss with growth, in both of which there was admittedly decomposing organic matter. It was also to be expected in the very rich soil. But it is doubtful whether, in the two cases of gain with growth, and therefore movement within the soil, and aëration of it, there would be any loss. In none of the experiments with loss, however, were the conditions comparable with those of ordinary soils, under ordinary treatment, and the losses found cannot be taken as any indication of what takes place in ordinary practice. It is probable that in such practice the loss by evolution of free nitrogen is much less than is generally assumed in discussions of this subject. Doubtless there is, however, frequently considerable loss by the drainage of nitrates.

FRANK considers that, independently of direct evidence against the supposition that the gains were due to the absorption of combined nitrogen from the atmosphere, an objection to such a view is that it would not explain the circulation of nitrogen in nature; and his main conclusion is, that there are two actions going on within the soil, one liberating nitrogen, and the other bringing it into combination, the latter favoured by vegetation.

Upon the whole it would seem that the losses found by FRANK may be explained by the special conditions of the experiments themselves; whilst the gains, if not to be accounted for by sources of error incidental to experiments made in free air, can only be explained by fixation in some way.

The most remarkable of the results indicating the fixation of free nitrogen are those of Professor HELLRIEGEL and Dr. WILFARTH. HELLRIEGEL found that whilst plants of the gramineous, chenopodiaceous, polygonous, and cruciferous families required combined nitrogen to be supplied within the soil, papilionaceous plants did not depend on such soil-supplies.

Peas sometimes grew luxuriantly in washed sand with nutritive solutions free from nitrogen, but sometimes failed, root-nodules being developed coincidently with luxuriance, but not without it. But when to the non-nitrogenous sandy matrix a few c.c. of the watery extract of a rich soil were added, the luxuriance was always marked, as also was the development of the root-nodules. Lupins, however, failed when treated in the same way, but succeeded when seeded by a watery extract of a

sandy soil where lupins were growing well, and root-nodules were then abundantly produced.

The amounts of produce recorded seemed to leave no doubt that they contained much more nitrogen than was supplied in the seed; whilst the amount added in the soil-extract was quite immaterial. The negative result with Gramineæ, with peas under sterilised conditions, or in sand not seeded with rich soil-extract, and with lupins in sand not seeded, or seeded with the rich soil-extract, and, on the other hand, the positive result with peas in the seeded sand, and with lupins when the sand was seeded with an extract from a suitable soil, seemed to exclude the supposition of any other source of gain than the fixation of free nitrogen under the influence of micro-organisms; and at first HELLRIEGEL was disposed to connect the action with the root-nodules and their contents.

WILFARTH gave the results of a subsequent season's experiments, which fully confirmed those recorded by HELLRIEGEL, both as to the negative result with other plants, and to the positive result with Papilionaceæ. Peas grew luxuriantly when the nitrogen-free soil was seeded with the watery extract from any cultivated soil, but serradella and lupins only when seeded with an extract from soil where these plants were growing.

In four experiments with lupins nearly 50 times as much dry substance was produced, and nearly 100 times as much nitrogen was assimilated, with, as without, seeding with the soil-extract!

WILFARTH concluded that the Papilionaceæ can derive the whole of their nitrogen from the air, but that it is doubtful whether the root-nodules are connected with the fixation, though the results point to the agency of bacteria in some way.

In reference to these results, whilst it can hardly be said that there is any unsolved problem in regard to the source of the nitrogen of other than our leguminous crops, it must be admitted that in spite of all the investigations and discussions of the last 50 years, the source of the whole of the nitrogen of these crops has not been satisfactorily explained by results obtained on the lines of inquiry until recently adopted. Evidence obtained on new lines should therefore receive careful consideration; and there can be no doubt that in recent years cumulative evidence has been adduced indicating that certain chlorophyllous plants may avail themselves of nitrogen brought into combination under the influence of lower organisms; the development and action of which would seem in some cases to be a coincident of the growth of the higher plants to be benefited. But such a conclusion is of such fundamental importance that further confirmation must yet be demanded before it can be considered to be fully established.

So long ago as 1853, Professor EMIL VON WOLFF obtained 6 times as much dry produce of clover, grown in an ignited rich meadow soil, as in the same soil in its natural state. Thus, the increased growth, and the increased assimilation of nitrogen, took place in a soil not only nitrogen-free, but sterilised; so that, unless micro-

organisms were acquired during growth, the supposition of their influence in fixing free nitrogen would be excluded.

Much more recently WOLFF has made numerous experiments with oats, potatoes, and various Papilionaceæ, in river-sand; in some cases unwashed, and in some washed; in some without manure, in some with purely mineral manure, and in some with nitrate in addition. Accordantly with common experience, there was little increase in the oats or potatoes with mineral, but much with nitrogenous manure; and, on the other hand, with the Papilionaceæ there was very marked increase with the mineral manure, and but little more by adding nitrate. In the experiments with lupins, beans, and clover, in unwashed sand, the results indicated gain of nitrogen beyond that probably due to the nitrogenous impurity in the sand; but with sand-peas, grown in washed sand, which was assumed to be nitrogen-free, the gains from some external source were unmistakable.

As to the explanation, WOLFF does not suppose that free nitrogen is fixed by the plants themselves; nor does he favour the view that it was fixed by the agency of micro-organisms. The plants may take up combined nitrogen from the air by their leaves; but he thinks it more probable that combined nitrogen is absorbed from the air by the soil, and that free nitrogen is fixed within the soil under the influence of porous and alkaline bodies. He admits that it is not explained why cereals do not benefit by these actions as well as Papilionaceæ; and he suggests whether the greater evaporation from their leaves causes greater aëration of the soil.

Here, then, the gain of nitrogen by the Leguminosæ is explained in a very different manner from that assumed by other recent experimenters. It seems to us, however, that the undoubted fact that the Gramineæ, and other plants than the Papilionaceæ, do not benefit by the actions supposed, excludes the supposition that WOLFF's results with Papilionaceæ are to be so explained. It is true that neither in the growth of the clover in ignited soil, nor in that of the sand-peas in the washed sand, were the conditions such as would seem favourable for the presence, development, and agency of micro-organisms. But if, in the experiments in free air, there was no accidental source of combined nitrogen, it would seem that the influence of micro-organisms is at least as probable as that of the actions which WOLFF supposes.

Professor ATWATER made numerous experiments, both on the germination and on the growth of peas. In eleven out of thirteen experiments on germination more or less loss of nitrogen was observed. In all but one out of fifteen experiments on vegetation, there was a gain of nitrogen, which was very variable in amount, and sometimes very large. As a general conclusion, he states that in some of the experiments half or more of the total nitrogen of the plants was acquired from the air.

He considers that germination without loss of nitrogen is the normal process; that loss, whether during germination or growth, is due to decay, and therefore only accessory. Nevertheless, he goes into calculations of some of his own results, showing, by the side of the actual gains, the greater gains supposing there had been

a loss of 15 per cent. of nitrogen, and the still greater gains if there had been a loss of 45 per cent., as in an experiment by BOUSSINGAULT under special conditions. Further, he says that whilst actually observed gains are proof of the acquisition of nitrogen, the failure to show gain only proves non-fixation, if it be proved that there was no liberation. He suggests that the negative results obtained by BOUSSINGAULT and at Rothamsted may be accounted for by liberation; though at the same time he recognises that the conditions of the experiments excluded the action of either electricity or microbes. We may remark that, in the experiments both of BOUSSINGAULT and at Rothamsted, any cases of decay were carefully observed, and the losses found explained accordingly; and it may be confidently asserted that the conclusions drawn were not vitiated by any such loss. This specious objection, putting out of court all negative results, is, however, a very old one; as also is the one resuscitated by ATWATER, that luxuriance must be forced to a certain degree to favour the fixation of free nitrogen. On this point we may state that the results obtained at Rothamsted were as distinctly negative when luxuriance was favoured by supplies of combined nitrogen as when it was not.

ATWATER concludes that his results do not settle whether the nitrogen gained was acquired as free or combined nitrogen, by the foliage, or by the soil. He considers, however, that, in his experiments, the conditions were not favourable for the action either of electricity or of micro-organisms; and he favours the assumption that the plants themselves were the agents. Lastly, he considers the fact of the acquisition of free nitrogen in some way to be well established; and that thus facts of vegetable production are explained, which otherwise remain unexplained. To this, and other points involved, we shall refer again in our concluding remarks.

Lastly, we have to summarise those of the results and conclusions of BOUSSINGAULT which bear upon the present aspect of the question of the sources of the nitrogen of vegetation. In his earlier experiments, as in those at Rothamsted, sterilised materials had been used as soils; but in 1858 he commenced a series in which more or less of a rich garden soil was mixed with sand and quartz. In some cases the plants were grown in free air, and in others in closed vessels with confined air. In several cases there was more or less gain of nitrogen; but the greatest gain was in an experiment with a lupin grown in a closed vessel. BOUSSINGAULT points out that it was the soil and not the plant that had fixed the nitrogen. The result was so marked that he repeated the experiment in 1859, when he obtained almost identically the same amount of gain as in 1858. He also put 120 grams of the rich soil into a shallow dish, moistened it with distilled water, and exposed it to the air as an experiment on fallow. The results showed a small gain of nitrogen.

BOUSSINGAULT further found that mycodermic vegetation went on in rich soil, and he considered the gains of organic nitrogen represented the remains of such vegetation; whilst the fallow experiment indicated that the experimental plants had little to do with the action. His general conclusion was, that from the numerical results

it must be believed that the soil had fixed nitrogen; and he considered that, if there were not absolute proof, there was strong presumption, that the nitrogen of the air takes part in nitrification.

In the next year, 1860, he put into one large glass balloon a mixture of rich soil and sand, and into another a similar mixture with cellulose in addition; each was moistened with distilled water, and the vessels were then closed up for 11 years. During this period, without cellulose rather more, and with cellulose rather less, than one-third of the nitrogen of the soil was nitrified; but in neither case was there any gain of total combined nitrogen. There was, indeed, in both cases, a slight loss of nitrogen indicated. Boussingault concluded that free nitrogen had not contributed to the formation of nitric acid.*

The later results of Boussingault did not therefore confirm those he obtained in 1858 and 1859; and in answer to one of ourselves he wrote in 1876, that he was not aware of any irreproachable observation which established the reality of the fixation of free nitrogen by the soil. He further stated his belief that neither the higher plants, nor mycoderms, nor fungi (*champignons*), fix free nitrogen. He also maintained the same view in conversation in 1883.

Boussingault's very distinct final conclusion against the supposition of the fixation of free nitrogen within the soil, by the agency of the lower organisms, notwithstanding his own clear recognition in 1858 and 1859 of the possibility of such an action, points to the necessity for still further confirmation of the evidence of others on the point during the last few years; for it will be remembered that whatever other sources of error were possible, the experiments in question were made in closed vessels, and not in free air, with all the risks incident to experiments so conducted; and if there may have been error with such an experimenter, and under such conditions, caution should surely be exercised in accepting very important conclusions founded on results obtained for the most part under less favourable conditions.

3. *General Considerations and Conclusions.*

So much for the evidence of direct experiment as to whether the higher plants, or soils, by the agency either of micro-organisms or otherwise, fix the free nitrogen of the atmosphere. It is clear that since experimenting in free air instead of in closed vessels has become more general, there has been a great accumulation of evidence which is held to show the fixation of free nitrogen. But not only are the gains in

* Quite recently ('Compt. Rend.,' vol. 106, 1888, pp. 805 and 898) M. Schlœsing referring to these results says that for his part he was satisfied with this result of Boussingault, and should not have entered upon new experiments, had not the question been recently taken up and answered in a contrary sense. He then gives the results of experiments in which he submitted various soils to the action of air in closed vessels, supplying oxygen as it was used up. The result was that the air of the vessels neither lost nor gained nitrogen. There was therefore no fixation.

some cases small, and in others very large, but the modes of explanation are so different, indeed so conflicting, that it seems essential to hold final judgment in abeyance for the present.

The various modes of explanation of the observed gains of nitrogen are:—that combined nitrogen has been absorbed from the air, either by the soil or by the plant; that there is fixation of free nitrogen within the soil by the agency of porous and alkaline bodies; that there is fixation by the plant itself; that there is fixation within the soil by the agency of electricity; and finally that there is fixation under the influence of micro-organisms within the soil. The balance of the evidence recorded, is undoubtedly in favour of the last-mentioned mode of explanation. Indeed, it seems to us that, if there be not experimental error, there is fixation within the soil, under the influence of micro-organisms, or other low forms.

Assuming that definite decision on the point must wait for further evidence and discussion, it will nevertheless be well, in the meantime, to consider the facts of agricultural production in their bearing on the question, with a view of forming a judgment as to how far the establishment of the reality of the fixation of free nitrogen, either by the plant or by the soil, is so essential for the solution of the problems which such production presents, as is by some supposed.

It has been seen that much of the investigation that has been undertaken in recent years, has been instigated by the assumption that there must exist natural compensation for the losses of combined nitrogen which the soil suffers by the removal of crops, and for the losses which result from the liberation of free nitrogen from its combinations under various circumstances. In some cases, however, the object seems to have been for the most part limited to an attempt to solve the admitted difficulty as to the explanation of the source of the whole of the nitrogen of the Leguminosæ.

As to the losses which the soil sustains by the removal of crops, BERTHELOT for example assumes that 50 to 60 kilog. of nitrogen will be annually removed from a hectare of meadow (= 45 to 54 lbs. per acre), and that as only 10 kilog., or less, of this will be restored as combined nitrogen in rain, &c., there will be an annual loss of from 40 to 50 kilog. per hectare (= 36 to 45 lbs. per acre); so that, if there were not compensation from the free nitrogen of the air, the soil would become gradually exhausted. Further, he considers that the fact of the fixation of free nitrogen, not only explains how fertility is maintained, but how argillaceous soils which are sterile when first brought into contact with the air, gradually yield better crops, and at length become vegetable moulds. FRANK again, assumes that the average loss of nitrogen by the removal of crops is 51 kilog. per hectare (= 45 lbs. per acre).

It is quite true, that a good hay crop may contain as much as 50 to 60 kilog. of nitrogen per hectare, but it may safely be affirmed that, in ordinary practice, even in the case of an unusually fertile meadow, such an amount is not annually removed for a number of years in succession, without the periodical return of manure supplying nitrogen; whilst, taking the average of soils, the annual yield will not reach the

amount supposed, even with the ordinary periodic return, and without such return gradual exhaustion would be very marked. Indeed, it is well known that there is no more exhausting practice than the annual removal of hay without return of manure; so that, in point of fact, restoration in anything like the degree supposed certainly does not take place. Next to the removal of hay, the consumption of grass for the production of milk is the most, but still very much less, nitrogen-exhausting; whilst if the grass be consumed by store or fattening animals, the loss is very much less still; indeed it is very small.

Obviously, however, it is more important to consider, what is the probable average loss of nitrogen over a given area by the removal of crops generally, and not by that of grass alone. Moreover, in making such an estimate it is not the total nitrogen of the crops that has to be reckoned; but, taking into account the return by manure, only the amount eventually lost to the soil. With the great variation according to circumstances, it is of course very difficult to estimate this at all accurately; but we may state that two independent modes of estimate lead to the conclusion that, for Great Britain for example, the average annual loss of nitrogen is more probably under than over 20 lbs. per acre (= 22·4 kilog. per hectare). In fact, the loss by cropping, under the usual conditions of more or less full periodical return by manure, is by no means so great as is generally assumed in discussions of this subject.

The loss of nitrates by drainage may, however, in some cases be considerable. There may also, under some circumstances, be loss from the soil by the evolution of free nitrogen. Such loss may take place in the manure heap, or in soils very heavily manured, as in market gardening, for example. But in ordinary agriculture such excessive manuring seldom takes place; and the soil is generally much poorer in nitrogen than in the cases of the experiments which have been quoted as showing great loss from rich soils. Loss may also take place when the soil is deficiently aërated; but here again the conditions of the experiments cited, in which considerable loss by evolution of free nitrogen was observed, are not the usual conditions of soils in actual practice. Indeed, the balance of evidence is against the supposition that there is a constant and considerable loss by the evolution of free nitrogen from arable soils which are only moderately rich in organic nitrogen, and which are fairly drained, either naturally or artificially. Some illustrations bearing upon this point will be found at pages 62-3.

Again, M. Berthelot thinks it probable, though not absolutely established, that there is loss of nitrogen from the plant itself during growth. Long ago, we ourselves supposed that there was such loss; but careful consideration of the evidence relating to the subject has led us to conclude that it is not proved, and to believe that it probably does not take place. It may be observed that when in his vegetation experiments M. Boussingault found a loss of nitrogen, there was coincidently some decaying vegetable matter, such as fallen leaves; and in somewhat parallel experiments at Rothamsted, no loss of nitrogen was found as a coincident of growth, and in the

absence of dead vegetable matter. Indeed, if there were such loss during growth when there was no decay, either in M. BOUSSINGAULT's experiments or in our own, it must have been almost exactly balanced by corresponding gain; an assumption which is without any proof, but which has nevertheless had its advocates.

In fact we conclude, that under the existing conditions of practical agriculture in temperate climates, the annual loss of combined nitrogen over a given area, by cropping and otherwise, is by no means so great as has been assumed; that the restoration required to compensate the loss is therefore correspondingly less; and further, that the known facts relating to the maintenance or the reduction of the fertility of soils, do not point to the conclusion that such loss as actually does take place, is compensated by such restoration.

The well-known accumulation of nitrogen which takes place in the surface soil within a few years, when arable land is laid down to grass, is, it may be admitted, not conclusively explained. At the same time, there is, to say the least, quite as much evidence in favour of the assumption of a subsoil, as of an atmospheric, source. At Rothamsted, for example, there is, in soil and subsoil, to the depth at which the action of some deep-rooted and large nitrogen-accumulating plants has been proved, a store of about 20,000 lbs. of already combined nitrogen per acre. It is true that whilst many other soils and subsoils will contain as much, or more, many will contain much less. Still, if further investigation should confirm the indications given in this and former papers, that in the case of the deep and strong rooting, and high nitrogen-yielding, Leguminosæ, much at any rate of their nitrogen probably has its source in the combined nitrogen of the subsoil, and that the accumulation in the surface soil is due to nitrogenous crop-residue, the nitrogen of which has come from the subsoil, it is obvious that a like explanation would be applicable to the accumulation which takes place when arable land is laid down to grass, including herbage of various root-ranges, and various habits of root-collection.

Then, again, as to the supposition that the gains of nitrogen in argillaceous matters of very low initial nitrogen contents, which gains are attributed to the fixation of free nitrogen, serve to explain the gradual formation of vegetable soils, there cannot be any doubt that, so far as nitrogen is concerned, the natural fertility of most soils is due to the accumulation of ages of natural vegetation with little or no removal of it, by animals or otherwise. If the amounts of nitrogen even now brought into combination over a given area under the influence of electricity in Equatorial regions, were not exceeded in the earlier periods of the history of our globe, that would be quite sufficient, with growth and with little or no removal, through the ages which modern science teaches us to reckon upon, for the ascertained accumulations in natural prairie, or forest lands; and it is these which have to a great extent furnished us with our meadows and pastures, and arable soils. Frequently the natural forests have been on the more elevated, or the more undulating lands, and the soils they have formed

are less rich than the prairie lands, for the most part found in the valleys or on the plains. Taking the vast areas of fertile natural prairie on the American continent, for example, sometimes of several feet in depth, it may be estimated that, in such cases, each foot of depth will contain from 6000 to 10,000 lbs., or even more, of combined nitrogen per acre (= 6720 to 11,200 kilog. per hectare); and the probable time of these accumulations entirely obviates the necessity of calling in the aid of the free nitrogen of the atmosphere, brought into combination either under the influence of the plants themselves or of micro-organisms within the soil.

Further, the history of agriculture so far as it is known, indicates that soils under cultivation without supplies by manure from external sources, do, as a matter of fact, gradually become less fertile. This, as a rule, will take place more rapidly in undulating or high forest lands, than in the natural grass or prairie lands of the plains.

Again, if we compare the amount of nitrogen in the surface soil of permanent grass land, with that of adjoining land of the same original character, but which has for some time been under arable culture, we find that the latter is much poorer in nitrogen. In illustration, it may be stated that whilst the surface soil of the grass land at Rothamsted contains from 0·25 to 0·30 per cent. of nitrogen, that of the corresponding arable land only contains from 0·1 to 0·15 per cent. The arable soil has, in fact, originally been covered with natural vegetation of some kind, with comparatively little removal, and consequent accumulation; whilst, under arable culture, much of the accumulated nitrogen has been used up, and the loss has not been compensated by free nitrogen brought into combination, under the influence either of electricity, or of organisms within the soil. Whether or not there is any restoration of the kind supposed, we believe that a consideration of the origin of soils generally, and of the history of agriculture in different countries, will lead to the conclusion that the losses of combined nitrogen by cropping, and in other ways, are not compensated by corresponding amounts of free nitrogen constantly brought into combination.

The Rothamsted field experiments have indeed now been continued long enough to afford some pertinent examples bearing upon this subject.

Thus, in the case of the fields under continuous wheat, continuous barley, alternate wheat and fallow, and continuous root-crops, the average annual yield of nitrogen in the crops with mineral, but without nitrogenous manure, has only been about or under 20 lbs. per acre (= 22·4 kilog. per hectare); the amount has declined to less than the average in the later years, and, coincidently with the continuous and diminishing growth, the percentage of nitrogen in the surface soil has been considerably reduced. The loss by the removal of even such small crops, together with that by drainage, has, therefore, as a matter of fact, not been compensated by free nitrogen brought into combination, either by the plants, or within the soil.

In a field where the leguminous crop, beans, had been grown 25 years out of 32, with mineral but without nitrogenous manure, and had yielded less than average agricultural crops, the percentage of nitrogen in the surface soil was also greatly reduced.

In another field, where the leguminous crop, red-clover, had been sown 12 times in 30 years, the clover failed many times, the yield of nitrogen in the crops very greatly diminished, and the percentage of nitrogen in the surface soil was greatly reduced.

Again, in a rich garden soil, where red clover has been grown for more than thirty consecutive years, and has yielded throughout good, but gradually much diminishing crops, it was found, after the first 22 years, that the nitrogen in the surface soil had been reduced from 0·5095 to 0·3634 per cent., calculated on the soil dried at 100° C.

Even in an actual course of rotation, of turnips, barley, clover or beans, and wheat, with mineral, but without nitrogenous manure, the percentage of nitrogen in the surface soil has been much reduced; whilst in a parallel rotation in which fallow takes the place of the clover or beans, the reduction is still greater.

Thus, in all the cases cited, including gramineous, cruciferous, chenopodiaceous, and even leguminous crops, and a rotation of crops, when each has been grown for many years in succession without nitrogenous manure, and has yielded comparatively small and declining amounts of nitrogen in the crops, there has, coincidently, been a considerable reduction in the amount of nitrogen in the surface soil. There has, in fact, not been compensation from the free nitrogen of the air, or at any rate not at all in amount corresponding to the annual losses.

Lastly, grass land which, under the influence of a full mineral manure, including potash, but without any supply of nitrogen for more than thirty years, has grown crops containing large amounts of comparatively superficially rooting leguminous herbage, succeeded by increased amounts of gramineous herbage, has, under those conditions, yielded about the same amount of nitrogen per acre as M. Berthelot assumes to be the average produce of a meadow; but it has done so only with coincident great reduction in the nitrogen of the surface soil.

Whether, therefore, we consider the facts of agriculture generally, or confine attention to special cases under known experimental conditions, the evidence does not favour the supposition that a balance is maintained by the restoration of nitrogen from the large store of it existing in the free state in the atmosphere. Further, our original soil-supplies of nitrogen are, as a rule, due to the accumulations by natural vegetation, with little or no removal, over long periods of time; or, as in the case of many deep subsoils, the nitrogen is largely due to vegetable and animal remains, intermixed with the mineral deposits. The agricultural production of the present age is, in fact, so far as its nitrogen is concerned, mainly dependent on previous accumulations; and as in the case of the use of coal for fuel there is not coincident and corresponding restoration, so in that of the use or waste of the combined nitrogen of the

soil, there is not evidence of coincident and corresponding restoration of nitrogen from the free to the combined state.

In the case of agricultural production for sale, without restoration by manure from external sources, a very important condition of the maintenance of the amount of nitrogen in the surface soil, or of the diminished exhaustion of it, is the growth of plants of various range and character of roots, and especially of leguminous crops. Such plants, by their crop-residue, enrich the surface soil in nitrogen. It is, as a rule, those of the most powerful root-development that take up the most nitrogen from somewhere; and this fact points to a subsoil source. But independently of this, which obviously might be held to be only evidence of the necessity of obtaining water and mineral matters from below, in amount commensurate with the capability of acquiring nitrogen from the air, the experimental results recorded in this paper can leave little doubt that such plants obtain at any rate much of their nitrogen from the subsoil. The question remains—whether or not the whole of it is derived from the soil and subsoil? At present it is not proved that it is. It is equally not yet conclusively proved that it comes from the atmosphere. It may be safely affirmed that, in the case of our gramineous, our cruciferous, our chenopodiaceous, and our solaneous crops, atmospheric nitrogen is not the source. If, therefore, it should be proved to be the source in the case of the Leguminosæ, it may be that the development of the organisms capable of bringing free nitrogen into combination within the soil is favoured by leguminous growth and crop-residue, as there can be little doubt is the case with those which induce nitrification.

Bearing in mind, however, the very large store of already existing combined nitrogen, especially in subsoils, it is obviously important to consider, in what way, or in what degree, this store may contribute to chlorophyllous vegetation?

There is in the first place the question, whether the roots of some plants, and especially those of certain deep and powerfully rooting Leguminosæ, whose root-sap is strongly acid, may either directly take up organic nitrogen from the soil and subsoil, or may attack and liberate it for further change, the nitrogen so becoming more available.

Again, so far as is known, the Fungi generally, derive their nitrogen largely, if not exclusively, from organic nitrogen. In the case of those of fairy rings for example, there can be no doubt that they take up from the soil organic nitrogen which is not available to the meadow plants, and that, on their decay, their nitrogen becomes available to the associated herbage. In the case of the fungus-mantle observed by Frank on the roots of certain trees, it is to be supposed that the fungus takes up organic nitrogen, and so becomes the medium of the supply of the soil-nitrogen to the tree. More pertinent still, is the action of the nitrifying organisms in rendering the organic nitrogen of the soil and subsoil available to the higher plants. It may well be supposed, therefore, that there may be other cases in which lower organisms bring the

organic nitrogen of the soil and subsoil into a more available condition; whilst it seems not improbable that the growth and crop-residue of certain plants favour the development and action of special organisms. In conclusion we would submit that, whether or not it may eventually be conclusively proved that lower organisms have the power of bringing free nitrogen into combination within the soil, it would at any rate be not inconsistent with well established facts, were it found that the lower serve the higher, chiefly, if not exclusively, by bringing into a condition available to them, the combined nitrogen already existing, but in a comparatively inert state, in our soils and subsoils.

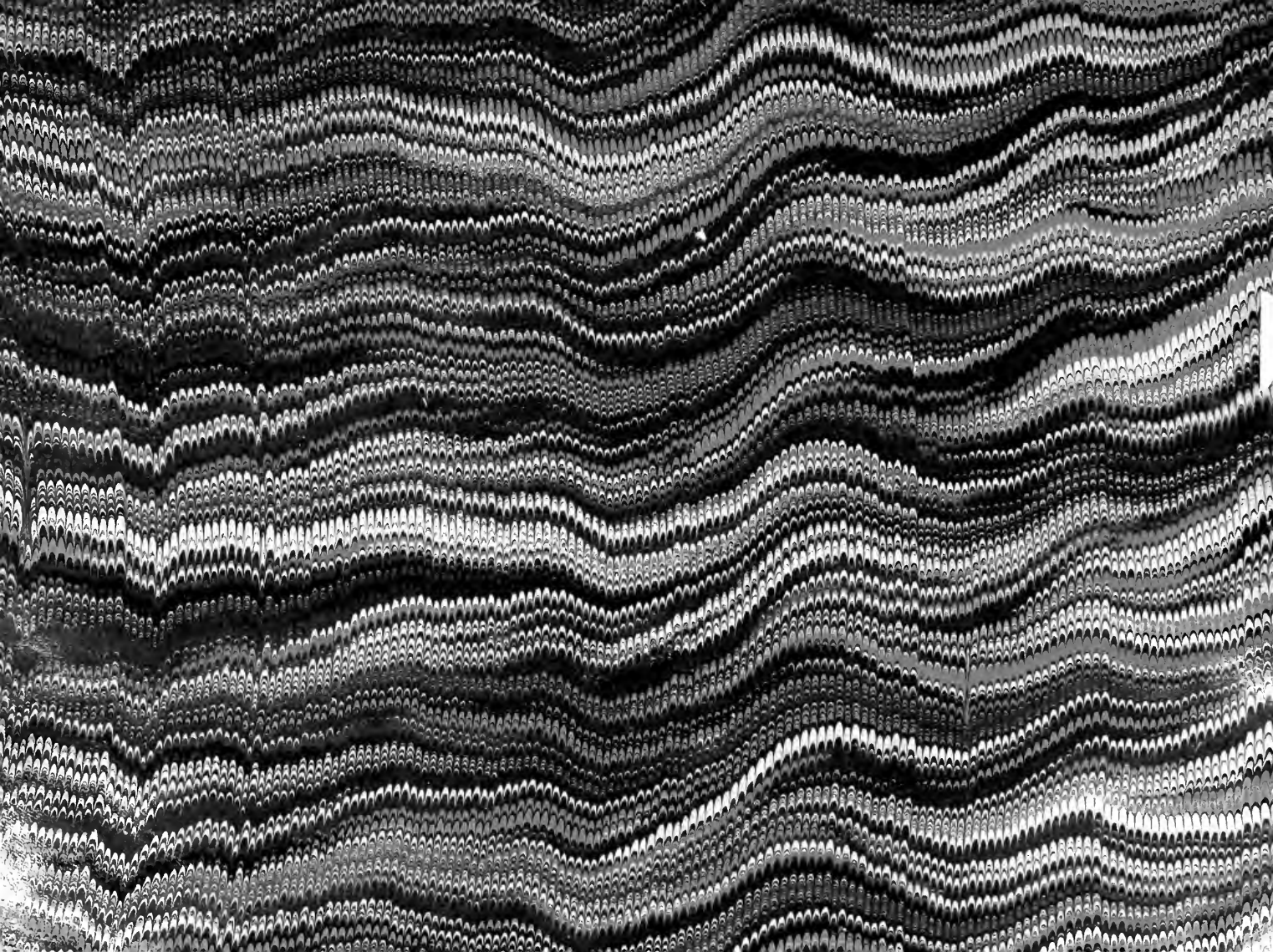
Postscript.

(Added October, 1888.)

As it seemed to us that, of the various results which have been considered relating to the fixation of free nitrogen, those of Hellriegel and Wilfarth are the most definite and significant, we decided to institute experiments on somewhat similar lines. We hoped to commence them early in the summer, but were not able to do so until the beginning of August. Decisive results cannot, therefore, be expected this season, but the experience gained will be of value in subsequent experiments.

This preliminary series comprises experiments with peas, blue lupins, and yellow lupins. The peas are grown (1) in washed sand, with the necessary mineral nutriment added, but with no supply of combined nitrogen beyond a small determined amount in the washed sand, and that in the seed sown; (2) in similarly prepared sand, but seeded with the extract from a rich garden soil; (3) in the rich garden soil itself. Each description of lupin is also grown—(1) in sand prepared as for the peas; (2) in the same washed sand, seeded with the extract from a sandy soil where lupins had grown luxuriantly; (3) in the lupin sandy soil itself; (4) in rich garden soil. The pots are all arranged in a small greenhouse.

As the plants are still growing, no quantitative results are as yet available. It may be observed, however, that, so far as can be judged by the eye, there seems, in the case of the peas, to be somewhat more growth where the sand was seeded with the soil-extract, supposed to contain organisms, than where it was not; whilst in the cases of the lupins there is apparently even somewhat less growth with than without the sandy soil-extract. Both with the peas and the lupins, the growth is very much more luxuriant in the garden soil; and in the case of the yellow lupins it is almost as luxuriant in the sandy soil in which lupins had grown, as in the garden soil. These first experiments can obviously be only considered as initiative; but it is intended to analyse the products in due course, and to undertake a new series earlier in the season next year.



Author 51033
Title (From Philosophical transactions) 4to
1(1861-69)

University of Toronto
Library

DO NOT
REMOVE
THE
CARD
FROM
THIS
POCKET

Acme Library Card Pocket
LOWE-MARTIN CO. LIMITED